

## Interaction of twenty-two carbazole derivatives with M<sub>1</sub>-muscarinic receptor using a theoretical model

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### Abstract

Several drugs have been used to treat asthma diseases, such as salmeterol, ipratropium bromide, montelukast, and fluticasone; however, some of these drugs can cause side effects such as hypokalemia, lactic acidosis, and hypotension. Analyzing these data, this study aimed to evaluate the possible interaction of twenty-two carbazole derivatives with the M<sub>1</sub>-muscarinic receptor to provide a new therapeutic alternative against asthma. The theoretical interaction of carbazole derivatives with M<sub>1</sub>-muscarinic receptor surface was determined using 5cxv protein, pirenzepine, atropine, AF-150, and PD159714 drugs as theoretical tools in a DockingServer software. The results showed differences in the interaction of carbazole derivatives with the 5cxv protein surface compared with pirenzepine, atropine, AF-150, and PD159714 drugs. Besides, constant inhibition (K<sub>i</sub>) for carbazole derivatives 11 and 22 was lower than for pirenzepine and AF-150 drugs. Other data indicate that K<sub>i</sub> values for 11 and 22 were higher than atropine and ipratropium bromide. In addition, the K<sub>i</sub> values for compounds 17 and 20 were like both atropine and PD150714 drugs. Finally, K<sub>i</sub> values for carbazole derivatives 17 and 20 were lower than pirenzepine, ipratropium bromide, and AF-150 reagents. All these data suggest that carbazole derivatives 11, 17, 20, and 22 may act as M<sub>1</sub>-muscarinic receptor inhibitor agents; this phenomenon could result in the regulation of bronchial tone in asthma disease.

**Keywords:** carbazole, derivatives, M<sub>1</sub>-muscarinic, receptor, asthma, docking.

## Interação de vinte e dois derivados de carbazol com receptor muscarínico M<sub>1</sub> utilizando modelo teórico

### Resumo

Vários medicamentos têm sido utilizados para tratar a asma, como salmeterol, brometo de ipratrópio, montelucaste, fluticasona; entretanto, alguns desses medicamentos podem causar efeitos colaterais como hipocalemia, acidose láctica e hipotensão. Analisando esses dados, este estudo teve como objetivo avaliar a possível interação de vinte e dois derivados do carbazol com o receptor muscarínico M<sub>1</sub> para fornecer uma nova alternativa terapêutica contra a asma. A interação teórica dos derivados de carbazol com a superfície do receptor muscarínico M<sub>1</sub> foi determinada usando proteína 5cxv, pিরanzepina, atropina, AF-150 e drogas PD159714 como ferramentas teóricas em um software DockingServer. Os resultados mostraram diferenças na interação dos derivados do carbazol com a superfície da proteína 5cxv em comparação com os medicamentos pিরanzepina, atropina, AF-150 e PD159714. Além disso, a inibição constante (K<sub>i</sub>) dos derivados carbazol 11 e 22 foi menor em comparação com as drogas pিরanzepina e AF-150. Outros dados indicam que os valores de K<sub>i</sub> para 11 e 22 foram

maiores em comparação com atropina e brometo de ipratrópio. Além disso, os valores de  $K_i$  para os compostos 17 e 20 foram semelhantes aos medicamentos atropina e PD150714. Finalmente, os valores de  $K_i$  para os derivados de carbazol 17 e 20 foram menores em comparação com os reagentes piranazepina, brometo de ipratrópio e AF-150. Todos estes dados sugerem que os derivados de carbazol 11, 17, 20 e 22 podem actuar como agentes inibidores do  $M_1$ -muscarínico receptor; esse fenômeno poderia resultar na regulação do tônus brônquico na asma.

**Palavras-chave:** carbazol, derivados, muscarínico  $M_1$ , receptor, asma, docking.

## 1. Introduction

Asthma is a chronic disease characterized by difficulty breathing with episodes of wheezing and coughing (Rodrigo et al., 2004; Lee et al., 2022; Chan; Lipwort, 2022). Asthma is a common inflammatory condition affecting millions more globally (Desai; Oppenheimer, 2011). There are several risk factors for developing asthma, such as genetic mutations (Herrera et al., 2022; Wheatley et al., 2022; Liu et al., 2022; Koumpagiotti et al., 2023; Stikke et al., 2023), tobacco (Li et al., 2022), pollution (Chatkin et al., 2022), obesity (Reyes-Angel et al., 2022), and viral/bacterial infections (Kraft, 2000; Darveaux; Lemanske, 2014; Jartti; Gern, 2017; Mikhail; Grayson, 2019).

It is important to mention that different drugs have been used to treat this clinical pathology, such as salbutamol and fenoterol (Svedmyr, 1985; Marquez; Vale, 2022), montelukast and Zafirlukast (Adkins; Brogden, 1998; Nayak, 2004), ipratropium bromide (El-Khoury et al., 2023), and others. Nevertheless, some of these drugs can produce adverse effects such as hypokalemia (Scheinin, 1987), lactic acidosis, hypotension (Colombo et al., 2022), and depression (Haarman et al., 2017).

In search of new therapeutic alternatives, several drugs have been developed to treat asthma; for example, a study indicated that a pyrano[3,2-g]quinoline-2,8-dicarboxylic acid analog can be used as an antiallergic to treat asthma disease (Cairns et al., 1985). In addition, a series of 1-ryridylnaphthalene derivatives were prepared to control asthma through phosphodiesterase 4 inhibition using a guinea pig model (Ukita et al., 1999). Besides, a study showed the synthesis and biological activity of a series of 1-Aryl-2,3-bis(hydroxymethyl)naphthalene derivatives against asthma via phosphodiesterase 4 inhibition (Iwasaki et al., 1996).

Other data displayed the preparation of a 2-Amino-2-phenylethanol analog as a selective  $\beta_2$ -adrenoceptor agonist to treat asthma *in vitro* (Xing et al., 2022). Other studies displayed the preparation of an anti-asthmatic drug (fevipiprant) through of DP<sub>2</sub>-receptor inhibition (Sandham et al., 2017). In addition, a series of anti-asthmatic agents (indazole-5-carboxamide derivatives) were developed with biological activity on IRAK4 (interleukin-1 receptor-associated kinase 4) (Sabnis et al., 2022).

On the other hand, in the search for new drugs to treat asthma, a series of theoretical studies have been carried out to characterize the interaction of different compounds with some biomolecules involved in the development of asthma. In this way, a study showed the synthesis of an anti-asthmatic agent ((E)-1-(8-hydroxyquinolin-7-yl)-3-phenylprop-2-en-1-ones) and their possible interaction with the phosphodiesterase 4 enzyme using a theoretical model (Chabukswar et al., 2016). Other data showed the coupling of galangylin with PPAR $\gamma$  (peroxisome proliferator-activated receptor gamma) using the Discovery Studio program; these data suggest that galangilil could produce changes in the inflammatory responses involved in asthma disease (Henry et al., 2020).

In addition, a theoretical study was carried out to evaluate the possible interaction of different quinoline derivatives as phosphodiesterase 4 enzyme antagonists using QSAR (Quantitative Structure-Activity Relationship), 3D pharmacophore, CoMFA (Comparative Molecular Field Analysis), and CoMSIA (Comparative Molecular Similarity Indices Analysis) models (Gaurav; Singh, 2014). All these data show that several experimental and theoretical methods have been used to characterize the molecular mechanism involved in the biological activity produced by some compounds on some biomolecules involved in the development of asthma disease. However, these data are unclear; perhaps this phenomenon could be due to different experimental approaches or different chemical characteristics of each compound.

This investigation aimed to determine whether twenty-two carbazole derivatives could interact with  $M_1$ -receptor cholinergic, which is involved in the constriction process of peripheral airways in asthma (Struckmann et al., 2003).

## 2. Materials and Methods

Different carbazole analogs (Figure 1) were used to evaluate their interaction M<sub>1</sub>-muscarinic receptor as follows:

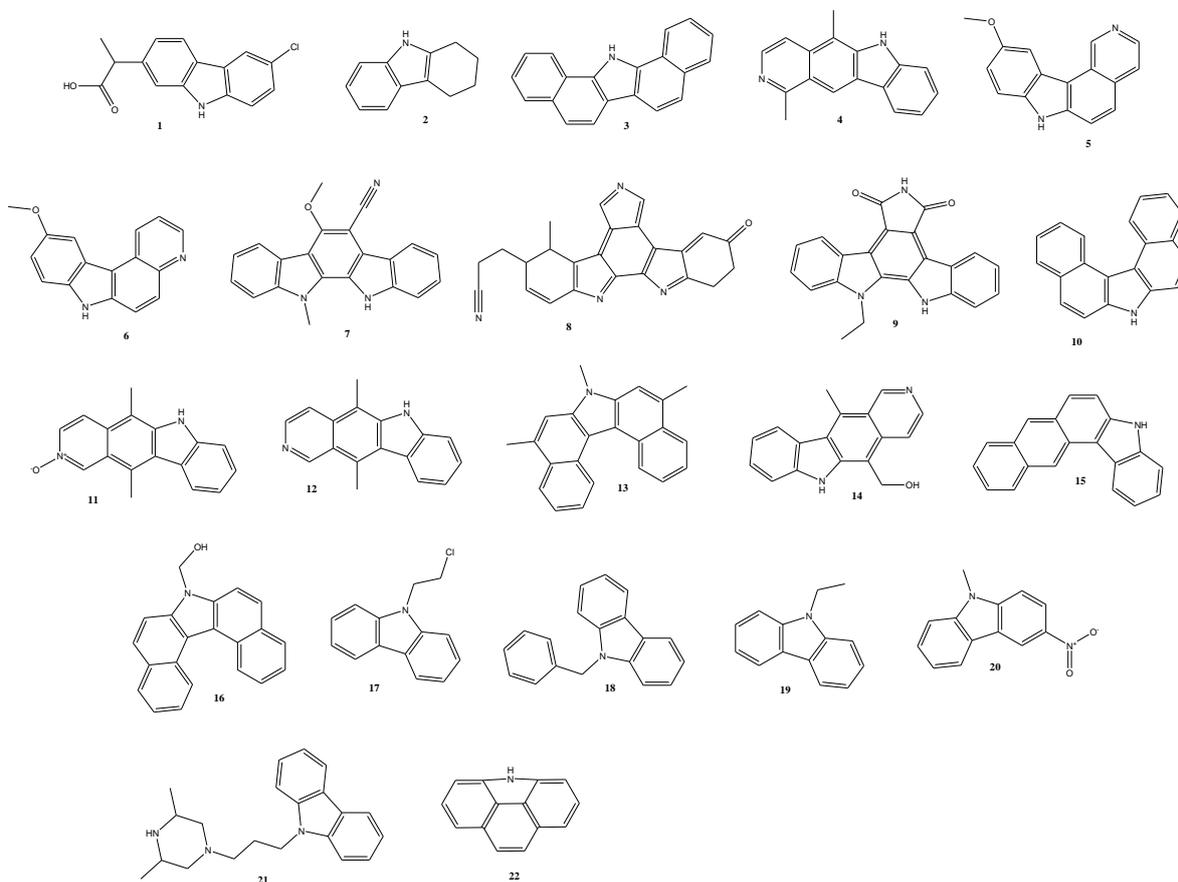


Figure 1. Chemical structure of carbazole derivative (1-22). Source: Authors, 2024.

1 = 2-(6-chloro-9H-carbazol-2-yl)propanoic acid

2 = 1,2,3,4-Tetrahydrocarbazole

3 = 1,2,7,8-Dibenzocarbazole

4 = 1,5-Dimethyl-6H-pyrido(4,3-b)carbazole

5 = 10-Methoxy-7H-pyrido[4,3-c]carbazole

6 = 10-Methoxy-7H-pyrido(2,3-c)carbazole

7 = 11,12-Dihydro-6-methoxy-11-methylindolo

(2,3-a)carbazole-5-carbonitrile

8 = 12-(2-Cyanoethyl)-6,7,12,13-tetrahydro-13-me-

thyl-5-oxo-5H-indolo(2,3-a)pyrrolo(3,4-c)carba-

zole

9 = 3-ethyl-3,13,23-triazahexacyclo[14.7.0.0.2,10.

0.4,9.0.11,15.

0.17,22]tricoso-1,4,6,8,10,15,17,19,

21-nonaene-12,14-dione

10 = 3,4,5,6-Dibenzocarbazole

11 = 5,11-Dimethyl-6H-pyrido(4,3-b)carbazole 2-oxide

12 = 5,11-Dimethyl-6H-pyrido(4,3-b)carbazole

13 = 5,7,9-Trimethyl-7H-dibenzo(c,g)carba-

zole

14 = 5-(Hydroxymethyl)-11-methyl-6H-

pyrido(4,3-b)carbazole

15 = 5H-Naphtho[2,3-c]carbazole

16 = 7-Hydroxymethyl-dibenzo(c,g)carbazole

17 = 9-(2-chloroethyl)carbazole

18 = 9-Benzyl-9H-carbazole

19 = 9-Ethyl-9H-carbazole

20 = 9-Methyl-3-nitro-9H-carbazole

21 = 9-[3-(3,5-Dimethyl-piperazin-1-yl)-pro-

pyl]-9H-carbazole

22 = 4H-Benzo[def]carbazole

### 2.1 Ligand-protein complex

Coupling of carbazole analogs with M<sub>1</sub>-muscarinic receptor was evaluated using 5cxv protein (Thal et al., 2016), pyranzepine, atropine, AF-150 (dimethyl-[2-[2-[4-(2-methylpropyl)phenyl]propanoyloxy]ethyl]-octylazanium),

and PD159714 (Hydroxy Lenalidomide) as theoretical tools in a DockingServer program. In addition, different types of binding energy involved in protein-ligand formation were determined.

### *2.2 Pharmacokinetics factors*

Pharmacokinetic parameters for carbazole analogs (11, 17, 20, and 22) were evaluated using the SwissADME software (Figuerola-Valverde et al., 2023).

### *2.3 Toxicity determination*

Possible toxicity produced by carbazole analogs 11, 17, 19, and 22 was determined using GUSAR software (Figuerola-Valverde et al., 2023).

## **3. Results and Discussion**

The objective of this study was to evaluate the interaction of twenty-two carbazole derivatives with the M1 muscarinic receptor as a therapeutic alternative to regulate bronchial tone in asthma disease as follows:

### *3.1 Docking analysis*

For several years, different theoretical methods have been developed that serve as tools for the synthesis or preparation of new drugs that interact with some biomolecules (Rarey et al., 1996; Jones et al., 1997; Österberg et al., 2002; Venkatachalam et al., 2003); in this way, the theoretical analysis on protein-ligand complex formation is necessary to predict the possible treatment of diseases.

This study aimed to evaluate the possible interaction of twenty-two carbazole derivatives with M1-muscarinic receptor cholinergic using the 5cxv-protein, pyranzepine, atropine, AF-150, and ipratropium bromide as theoretical tools in a DockingServer program. The results (Table 1) display different amino acid residues involved in the interaction of carbazole derivatives with 5cxv-protein surface compared with pyranzepine, atropine, AF-150, and ipratropium bromide; this phenomenon may be due to differences in binding energies levels involved.

### *3.2 Thermodynamic factors*

There are several mathematical methods (Friesner et al., 2004; Gökoğlu et al., 2023) and computer programs that have been used to predict some thermodynamic parameters such as free energy of binding, electrostatic energy, total intermolecular energy, and Van-Der Waals (vdW) + hydrogen bond (H-bond) + desolvation energy involved in the interaction of carbazole derivatives with some biomolecules (Shaikh et al., 2022; Patel., 2023). This study aimed to evaluate some theoretical thermodynamic parameters involved in the interaction of carbazole analogs (1 to 22) with M<sub>1</sub>-muscarinic receptor using 5cxv protein, pyranzepine, atropine, ipratropium bromide [muscarinic receptor non-selective inhibitors], (Augelli-Szafran et al., 1999; Zlotos et al., 1999), AF-150 [M<sub>1</sub>-muscarinic receptor selective agonist] (Fisher, 2000), and PD150714 [M<sub>1</sub>-muscarinic receptor selective antagonist] (Widzowski et al., 1997) as theoretical tools in DockingServer software.

The results (Table 2) displayed different thermodynamic parameters for carbazole derivatives compared with pyranzepine, atropine, ipratropium bromide, AF-150, and PD150714 reagents. Other data showed that the inhibition constant (K<sub>i</sub>) for carbazole derivatives 11 and 22 was lower than for pyranzepine and AF-150 drugs. Besides, the K<sub>i</sub> values for 11 and 22 were higher than atropine and ipratropium bromide. Other results indicate that K<sub>i</sub> for compounds 17 and 20 was similar to atropine and PD150714 drugs.

Finally, K<sub>i</sub> values for carbazole derivatives 17 and 20 were lower than pyranzepine, ipratropium bromide, and AF-150 reagents. All these data indicate that carbazole derivatives 11, 17, 20, and 22 could act as M<sub>1</sub>-muscarinic receptor inhibitor agents (Figure 2); this phenomenon could depend on the chemical characteristics of each carbazole derivative and the interaction with the different amino acid residues involved on the protein surface.

Table 1. Theoretical interaction of carbazole derivatives (compounds 1-22), pyranzepine, atropine, AF-150, ipratropium bromide, and PD159714 with 5cxv protein surface.

Compound	Aminoacid residues
Pyranzepine	Asp <sub>105</sub> ; Tyr <sub>106</sub> ; Ser <sub>109</sub> ; Gln <sub>110</sub> ; Trp <sub>157</sub> ; Thr <sub>189</sub> ; Ala <sub>193</sub> ; Ala <sub>196</sub> ; Phe <sub>197</sub> ; Trp <sub>378</sub> ; Tyr <sub>381</sub> ; Asn <sub>382</sub> ; Tyr <sub>404</sub> ; Cys <sub>407</sub> ; Tyr <sub>408</sub>
Atropine	Tyr <sub>106</sub> ; Ser <sub>109</sub> ; Gln <sub>110</sub> ; Trp <sub>157</sub> ; Thr <sub>189</sub> ; Ala <sub>196</sub> ; Phe <sub>197</sub> ; Trp <sub>378</sub> ; Tyr <sub>381</sub> ; Asn <sub>382</sub> ; Tyr <sub>404</sub> ; Cys <sub>407</sub> ; Tyr <sub>408</sub>
AF-150	Leu <sub>102</sub> ; Asp <sub>105</sub> ; Tyr <sub>106</sub> ; Ser <sub>109</sub> ; Tyr <sub>179</sub> ; Lau <sub>189</sub> ; Thr <sub>189</sub> ; Thr <sub>192</sub> ; Ala <sub>193</sub> ; Trp <sub>378</sub> ; Tyr <sub>381</sub> ; Asn <sub>382</sub> ; Val <sub>385</sub> ; Tyr <sub>404</sub> ; Cys <sub>407</sub>
Ipratropium bromide	Asp <sub>105</sub> ; Tyr <sub>106</sub> ; Ser <sub>109</sub> ; Gln <sub>110</sub> ; Val <sub>113</sub> ; Trp <sub>157</sub> ; Ala <sub>196</sub> ; Phe <sub>197</sub> ; Trp <sub>378</sub> ; Tyr <sub>381</sub> ; Asn <sub>382</sub> ; Tyr <sub>404</sub> ; Cys <sub>407</sub> ; Tyr <sub>408</sub>
PD150714	Tyr <sub>106</sub> ; Ser <sub>109</sub> ; Gln <sub>110</sub> ; Trp <sub>157</sub> ; Thr <sub>189</sub> ; Thr <sub>192</sub> ; Ala <sub>196</sub> ; Trp <sub>378</sub> ; Tyr <sub>381</sub> ; Asn <sub>382</sub> ; Tyr <sub>404</sub> ; Cys <sub>407</sub> ; Tyr <sub>408</sub>
1	Asp <sub>105</sub> ; Tyr <sub>106</sub> ; Ser <sub>109</sub> ; Trp <sub>157</sub> ; Leu <sub>183</sub> ; Thr <sub>189</sub> ; Thr <sub>192</sub> ; Trp <sub>378</sub> ; Tyr <sub>381</sub> ; Asn <sub>382</sub> ; Val <sub>385</sub> ; Tyr <sub>404</sub> ; Cys <sub>407</sub> ; Tyr <sub>408</sub>
2	Asp <sub>105</sub> ; Tyr <sub>106</sub> ; Ser <sub>109</sub> ; Gln <sub>110</sub> ; Trp <sub>157</sub> ; Ala <sub>196</sub> ; Trp <sub>378</sub> ; Tyr <sub>404</sub>
3	Asp <sub>105</sub> ; Tyr <sub>106</sub> ; Ser <sub>109</sub> ; Leu <sub>183</sub> ; Thr <sub>189</sub> ; Thr <sub>192</sub> ; Ala <sub>193</sub> ; Trp <sub>378</sub> ; Tyr <sub>381</sub> ; Asn <sub>382</sub> ; Tyr <sub>404</sub> ; Cys <sub>407</sub> ; Tyr <sub>408</sub>
4	Tyr <sub>106</sub> ; Ser <sub>109</sub> ; Thr <sub>189</sub> ; Ala <sub>193</sub> ; Trp <sub>378</sub> ; Tyr <sub>381</sub> ; Asn <sub>382</sub> ; Tyr <sub>404</sub> ; Cys <sub>407</sub>
5	Asp <sub>105</sub> ; Tyr <sub>106</sub> ; Ser <sub>109</sub> ; Gln <sub>110</sub> ; Trp <sub>157</sub> ; Ala <sub>196</sub> ; Phe <sub>197</sub> ; Trp <sub>378</sub> ; Asn <sub>382</sub> ; Tyr <sub>404</sub> ; Cys <sub>407</sub>
6	Asp <sub>105</sub> ; Tyr <sub>106</sub> ; Ser <sub>109</sub> ; Gln <sub>110</sub> ; Trp <sub>157</sub> ; Ala <sub>196</sub> ; Phe <sub>197</sub> ; Trp <sub>378</sub> ; Asn <sub>382</sub> ; Tyr <sub>404</sub> ; Cys <sub>407</sub>
7	Tyr <sub>106</sub> ; Ser <sub>109</sub> ; Gln <sub>110</sub> ; Trp <sub>157</sub> ; Leu <sub>183</sub> ; Thr <sub>189</sub> ; Thr <sub>192</sub> ; Ala <sub>196</sub> ; Trp <sub>378</sub> ; Tyr <sub>381</sub> ; Tyr <sub>404</sub> ; Cys <sub>407</sub> ; Tyr <sub>408</sub>
8	Tyr <sub>82</sub> ; Tyr <sub>85</sub> ; Leu <sub>86</sub> ; Trp <sub>101</sub> ; Leu <sub>102</sub> ; Tyr <sub>106</sub> ; Cys <sub>178</sub> ; Tyr <sub>179</sub> ; Leu <sub>183</sub> ; Tyr <sub>381</sub> ; Trp <sub>400</sub> ; Glu <sub>401</sub> ; Tyr <sub>404</sub>
9	Tyr <sub>106</sub> ; Ser <sub>109</sub> ; Gln <sub>110</sub> ; Val <sub>113</sub> ; Trp <sub>157</sub> ; Leu <sub>183</sub> ; Thr <sub>189</sub> ; Thr <sub>192</sub> ; Ala <sub>196</sub> ; Trp <sub>378</sub> ; Tyr <sub>381</sub> ; Tyr <sub>404</sub>
10	Asp <sub>105</sub> ; Tyr <sub>106</sub> ; Ser <sub>109</sub> ; Trp <sub>157</sub> ; Ala <sub>196</sub> ; Phe <sub>197</sub> ; Trp <sub>378</sub> ; Tyr <sub>381</sub> ; Asn <sub>382</sub> ; Tyr <sub>404</sub> ; Cys <sub>407</sub> ; Tyr <sub>408</sub>
11	Tyr <sub>106</sub> ; Ser <sub>109</sub> ; Trp <sub>157</sub> ; Leu <sub>183</sub> ; Thr <sub>189</sub> ; Thr <sub>192</sub> ; Trp <sub>378</sub> ; Tyr <sub>381</sub> ; Tyr <sub>404</sub> ; Cys <sub>407</sub> ; Tyr <sub>408</sub>
12	Asp <sub>105</sub> ; Tyr <sub>106</sub> ; Ser <sub>109</sub> ; Gln <sub>110</sub> ; Trp <sub>157</sub> ; Ala <sub>196</sub> ; Trp <sub>378</sub> ; Tyr <sub>404</sub> ; Cys <sub>407</sub>
13	Ser <sub>109</sub> ; Gln <sub>110</sub> ; Trp <sub>157</sub> ; Thr <sub>189</sub> ; Thr <sub>192</sub> ; Ala <sub>193</sub> ; Ala <sub>196</sub> ; Trp <sub>378</sub> ; Tyr <sub>381</sub> ; Asn <sub>382</sub> ; Val <sub>385</sub> ; Tyr <sub>404</sub> ; Cys <sub>407</sub>
14	Asp <sub>105</sub> ; Tyr <sub>106</sub> ; Ser <sub>109</sub> ; Gln <sub>110</sub> ; Trp <sub>157</sub> ; Ala <sub>196</sub> ; Trp <sub>378</sub> ; Tyr <sub>381</sub> ; Asn <sub>382</sub> ; Tyr <sub>404</sub> ; Cys <sub>407</sub> ; Tyr <sub>408</sub>
15	Tyr <sub>106</sub> ; Ser <sub>109</sub> ; Trp <sub>157</sub> ; Thr <sub>189</sub> ; Thr <sub>192</sub> ; Ala <sub>193</sub> ; Ala <sub>196</sub> ; Tyr <sub>381</sub> ; Tyr <sub>404</sub> ; Cys <sub>407</sub> ; Tyr <sub>408</sub>
16	Tyr <sub>106</sub> ; Ser <sub>109</sub> ; Gln <sub>110</sub> ; Val <sub>113</sub> ; Thr <sub>189</sub> ; Thr <sub>192</sub> ; Ala <sub>196</sub> ; Phe <sub>197</sub> ; Trp <sub>378</sub> ; Tyr <sub>381</sub> ; Asn <sub>382</sub> ; Tyr <sub>404</sub>
17	Asp <sub>105</sub> ; Tyr <sub>106</sub> ; Ser <sub>109</sub> ; Gln <sub>110</sub> ; Ala <sub>196</sub> ; Trp <sub>378</sub> ; Tyr <sub>381</sub> ; Asn <sub>382</sub> ; Tyr <sub>404</sub> ; Cys <sub>407</sub> ; Tyr <sub>408</sub>
18	Asp <sub>105</sub> ; Tyr <sub>106</sub> ; Ser <sub>109</sub> ; Gln <sub>110</sub> ; Val <sub>113</sub> ; Trp <sub>157</sub> ; Ala <sub>196</sub> ; Trp <sub>378</sub> ; Tyr <sub>381</sub> ; Asn <sub>382</sub> ; Tyr <sub>404</sub> ; Cys <sub>407</sub> ; Tyr <sub>408</sub>
19	Asp <sub>105</sub> ; Tyr <sub>106</sub> ; Ser <sub>109</sub> ; Gln <sub>110</sub> ; Trp <sub>157</sub> ; Ala <sub>196</sub> ; Trp <sub>378</sub> ; Tyr <sub>404</sub> ; Cys <sub>407</sub>
20	Asp <sub>105</sub> ; Tyr <sub>106</sub> ; Ser <sub>109</sub> ; Gln <sub>110</sub> ; Trp <sub>157</sub> ; Ala <sub>196</sub> ; Phe <sub>197</sub> ; Trp <sub>378</sub> ; Asn <sub>382</sub> ; Tyr <sub>404</sub> ; Cys <sub>407</sub> ; Tyr <sub>408</sub>
21	Asp <sub>105</sub> ; Tyr <sub>106</sub> ; Ser <sub>109</sub> ; Gln <sub>110</sub> ; Trp <sub>157</sub> ; Thr <sub>189</sub> ; Thr <sub>192</sub> ; Ala <sub>193</sub> ; Ala <sub>196</sub> ; Trp <sub>378</sub> ; Tyr <sub>381</sub> ; Tyr <sub>404</sub> ; Cys <sub>407</sub> ; Tyr <sub>408</sub>
22	Asp <sub>105</sub> ; Tyr <sub>106</sub> ; Ser <sub>109</sub> ; Gln <sub>110</sub> ; Trp <sub>157</sub> ; Ala <sub>196</sub> ; Trp <sub>378</sub> ; Tyr <sub>404</sub>

Source: Authors, 2024.

Table 2. Thermodynamic parameters involved interacting phenanthroline derivatives (compounds 1-22), decernotinib, and tofacitinib with 5cxv protein surface using the DockingServer program.

Compound	A	B	C	D	E	F
Pyranzepine	-9.08	222.22	-9.16	-0.30	-9.46	783.07
Atropine	-8.01	1.36	-8.67	-0.22	-8.89	674.30
Ipatropium bromide	-7.68	2.33	-10.30	-0.21	-10.52	730.33
AF-150	-4.41	585.18	-8.61	-0.01	-8.62	1006.39
PD150714	-7.99	1.38	-10.35	0.10	-10.25	757.19
1	-8.34	766.41	-8.80	-0.13	-8.93	689.78
2	-6.99	7.50	-6.96	-0.03	-6.99	528.30
3	-8.24	919.26	-8.22	-0.02	-8.24	696.00
4	-8.73	400.49	-8.70	-0.02	-8.73	672.93
5	-8.32	792.57	-8.57	-0.06	-8.62	632.31
6	-8.36	740.72	-8.67	0.01	-8.66	631.11
7	-5.25	141.76	-5.86	-0.03	-5.89	754.85
8	-8.27	873.22	-8.80	0.04	-8.76	914.72
9	-0.72	297.94	-1.02	0.00	-1.02	762.35
10	-10.30	28.07	-10.27	-0.03	-10.30	643.52
11	-7.67	2.39	-7.64	-0.03	-7.67	674.13
12	-8.73	401.64	-8.69	-0.04	-8.73	614.89
13	-8.20	971.04	-8.19	-0.01	-8.20	747.18
14	-8.72	406.10	-8.76	-0.01	-8.77	626.38
15	-9.27	161.30	-9.26	-0.01	-9.27	693.56
16	-9.39	131.65	-9.94	-0.04	-9.98	717.23
17	-8.00	1.37	-8.53	-0.01	-8.54	591.56
18	-8.91	296.01	-9.05	-0.01	-9.67	647.88
19	-7.17	5.57	-7.46	-0.01	-7.47	569.22
20	-7.99	1.38	-8.39	0.10	-8.29	579.20
21	-9.97	48.80	-10.36	-0.99	-11.35	764.32
22	-7.49	3.22	-7.47	-0.03	-7.49	539.77

Note: **A** = Est: Free Energy of Binding (kcal/mol); **B** = Inhibition Constant, Ki (mM); **C** = vdW + Hbond + desolv Energy (kcal/mol); **D** = Electrostatic Energy (kcal/mol); **E** = Total Intermolec. Energy (kcal/mol); **F** = Interact. Surface; **ABS** = 4-(2-Aminoethyl) benzene-sulfonamide. Source: Authors, 2024.

For example, compound 11 could bind to amino acids Thr<sub>189</sub> and Thr<sub>192</sub> involved on the 5cxv protein surface through hydrogen bonding between the carboxyl of amino acid residues and nitro group for carbazole derivative (Figure 3). Other data suggest that compound 17 may bind to Gln110 aminoacid residue via hydrogen bonding with the chloride atom involved in the chemical structure of the carbazole derivative. Besides, the nitro group of carbazole analog (compound 20; Figure 4) could interact with both Tyr404 and Asp105 amino acid residues through hydrogen bonding. Finally, other results indicate that carbazole derivative 22 may be bound to Tyr106 aminoacid of 5cxv protein surface.

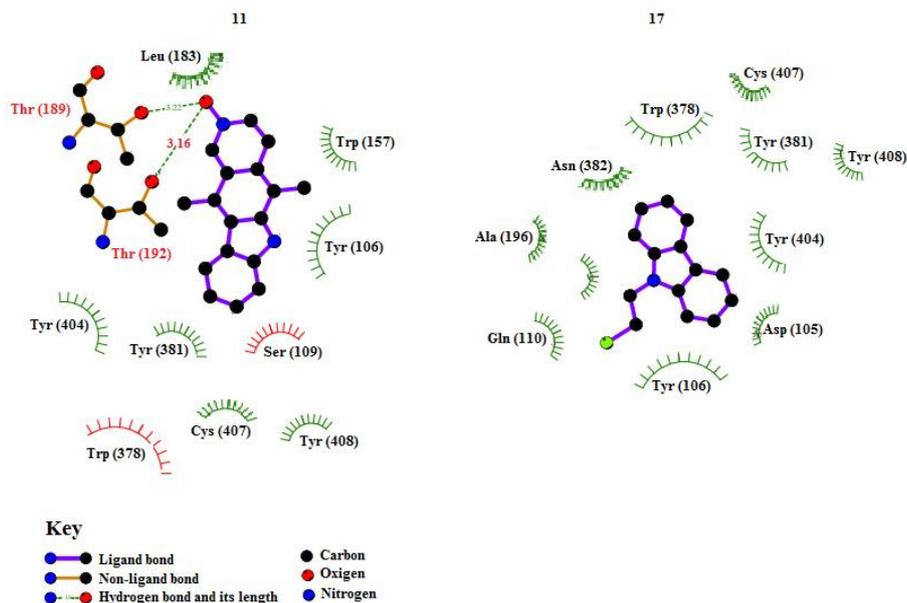


Figure 3. Interaction of carbazole derivatives 11, and 17 with 5cxv protein surface. I visualized with the DockingServer program. Source: Authors, 2024.

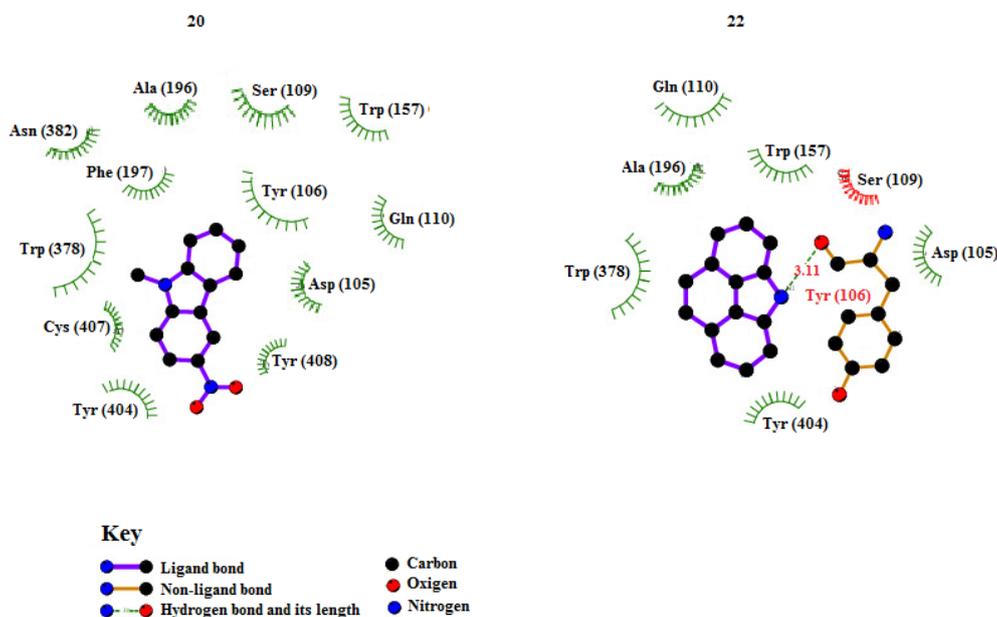


Figure 4. Coupling of carbazole analogs 20, and 22 with 5cxv protein surface. I visualized with the DockingServer program. Source: Authors, 2024.

### 3.3 Pharmacokinetic parameters

For several years, different theoretical methods have been used to predict some pharmacokinetics parameters involved in biological activity produced by some drugs such as ADME/PK (Eddershaw et al., 2000), SAAM II (Barrett et al., 1998) and SwissADME (Lauro et al., 2022) and others. Analyzing these data in this research, different pharmacokinetic parameters for carbazole derivatives 11, 17, 20, and 22 were determined using SwissADME.

Table 3 shows different pharmacokinetic parameters; the results suggest that compounds 11, 17, 20, and 22 may have higher gastrointestinal absorption and could act as CypA2 CypAC19 inhibitor agents. Furthermore, other

data indicate that carbazole derivative 17 could be a substrate of P-glycoprotein (P-gp is widely distributed and expressed in the intestinal epithelium where it pumps different xenobiotics); this phenomenon could decrease its bioavailability. All these data suggest that the gastrointestinal absorption degree depends on differences in the chemical structure, which could be conditioned by the lipophilicity degree that plays a very important role in the absorption and distribution of several drugs (Remko, 2007; Yang et al., 2012).

Table 3. Pharmacokinetics parameters determined for carbazole derivatives 11, 17, 20, and 22. Using the SwissADME program.

Parameter	Compounds			
	11	17	20	22
GI absorption	High	High	High	High
BBB permanent	Yes	Yes	Yes	Yes
P-gp substrate	Yes	No	No	No
CYP1A2 inhibitor	Yes	Yes	Yes	Yes
CYP2C19 inhibitor	Yes	Yes	Yes	Yes
CYP2C9 inhibitor	No	No	No	No
CYP2D6 substrate	No	Yes	No	No
CYP3A4 inhibitor	No	No	No	No
Concensus LogP <sub>OW</sub>	3.25	3.75	2.27	2.56

Source: Authors, 2024.

### 3.4 Toxicity analysis

Different theoretical programs have been developed to predict the toxicity degree of several drugs, such as Coral (Toropov et al., 2017), Virtual Tox Lab (Vedan et al., 2015), Topkat (Venkatapathy et al., 2004), and others. Analyzing these data in this study, the Gussar program (Figueroa-Valverde et al., 2023) was used to predict the possible toxicity produced by carbazole derivatives 11, 17, 20, and 22 through different routes of administration. The results suggest that carbazole derivative 11 could require higher doses to produce toxicity via intraperitoneal and oral routes compared to compounds 17, 20, and 22. Besides, the carbazole analog 22 requires a lower dose to produce toxicity through either Intraperitoneal or oral routes in comparison with compounds 11, 17, and 20 (Table 4). All these data indicate that the toxicity degree may depend on the chemical characteristics of each carbazole derivative.

Table 4. Rate acute toxicity predicted for carbazole derivatives (11, 17, 20, and 22) using GUSAR program.

Compound	Rat IP (mgkg)	Rat IV (mgkg)	Rat Oral (mgkg)	Rat SC (mgkg)
11	1064.00	49.20	2344.00	141.70
17	343.20	19.47	801.30	255.70
20	284.00	46.95	1506.00	226.50
22	216.70	49.80	722.10	542.00

Note: IP = Intraperitoneal route of administration (LD<sub>50</sub>); IV = Intravenous route of administration (LD<sub>50</sub>); Oral - Oral route of administration (LD<sub>50</sub>); SC = Subcutaneous route of administration (LD<sub>50</sub>). Source: Authors, 2024.

## 4. Conclusions

In this research, the possible interaction of some carbazole derivatives with the M<sub>1</sub>-muscarinic receptor is reported using the 5cxv protein as a theoretical tool with the aim of providing a therapeutic alternative for the treatment of asthma. The results suggest that carbazole derivatives 11, 17, 20 and 22 could act as M<sub>1</sub>-muscarinic receptor inhibitors; this phenomenon could result in the regulation of bronchial tone in asthma disease.

## 5. Authors' Contributions

*Figuroa Valverde Lauro*: article writing, data evaluation, and corrections. *López-Ramos Maria*: analysis data. *Rosas Nexticapa Marcela*: article writing, data evaluation, and corrections. *Alvarez-Ramirez Magdalena*: reading, writing, and evaluating data. *Mateu-Armad Maria Virginia*: analysis data. *Hau-Heredia Lenin*: data analysis. *Cauch-Carrillo Regina*: software usage, data analysis.

## 6. Conflicts of Interest

No conflicts of interest.

## 7. Ethics Approval

Not applicable.

## 8. References

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