Theoretical interaction of muscarinic receptor antagonist with vascular endothelial growth factor receptors (VEGF-R1, R_2 and R_3) as a therapeutic alternative to treat cancer

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Abstract

Several biomolecules have been the target of some drugs for the treatment of cancer; however, there is little information on the interaction of muscarinic antagonists with vascular endothelial growth factor receptor (VEGF-R1, R2, R3). The aim of this research was to determine the possible interaction of muscarinic antagonists such as atropine, ML381, af-dx 386, azaprophen, darifenacin, dicyclomine, PD-102807, pirenzepine, telenzepine, Zamifenacin, and cyclohexylamine with VEGF-R1, R2, and R3. The theoretical interaction of muscarinic antagonists with VEGF-R1, R2, and R3 was carried out using the 2ho4, 3hng, and 4bsj proteins as theoretical tools. Besides, cabozantinib, pazopanib, regorafenib, and sorafenib drugs were used as controls. The results showed differences in the number of aminoacid residues and energy levels involved in the interaction of muscarinic antagonists with 2ho4, 3hng, and 4bsj proteins compared with the controls. Besides, the inhibition constants (Ki) values for atropine, ML-381, zaniferacin, and dicyclomine were lower compared with some controls. In conclusion, the results suggest that atropine, ML-381, zaniferacin, and dicyclomine could act as VEGF receptor inhibitors, could result in changes in the biological activity of angiogenesis, and this phenomenon could be translated as a decrease in cancer cell growth. Therefore, these drugs could be a good therapeutic alternative to evaluate their biological activity in some cancer models.

Keywords: cancer, muscarinic, antagonists, angiogenesis.

Interação teórica do antagonista do receptor muscarínico com os receptores do fator de crescimento endotelial vascular (VEGF-R1, R2 e R3) como alternativa terapêutica para tratar o câncer

Resumo

Várias biomoléculas têm sido alvo de alguns medicamentos para o tratamento do câncer; Entretanto, há pouca informação sobre a interação dos antagonistas muscarínicos com os receptores do fator de crescimento endotelial vascular (VEGF-R1, R2, R3). O objetivo desta pesquisa foi determinar a possível interação de antagonistas muscarínicos como atropina, ML381, af-dx 386, azaprofeno, darifenacina, diciclomina, PD-102807, pirenzepina, telenzepina, zamifenacina e ciclo-hexilamina com VEGF-R1, R2 e R3. A interação teórica de antagonistas muscarínicos com VEGF-R1, R2 e R3 foi realizada usando as proteínas 2ho4, 3hng e 4bsj como ferramentas teóricas.

Além disso, os medicamentos cabozantinibe, pazopanibe, regorafenibe e sorafenibe foram usados como controles. Os resultados mostraram diferenças no número de resíduos de aminoácidos e níveis de energia envolvidos na interação dos antagonistas muscarínicos com as proteínas 2ho4, 3hng e 4bsj em comparação com os controles. Além disso, os valores das constantes de inibição (Ki) para atropina, ML-381, zaniferacina e diciclomina foram menores em comparação com alguns controles. Em conclusão, os resultados sugerem que a atropina, ML-381, zaniferacina e diciclomina podem atuar como inibidores do receptor VEGF, resultando em alterações na atividade biológica da angiogênese, e esse fenômeno pode ser traduzido como uma diminuição no crescimento de células cancerígenas. Portanto, esses medicamentos podem ser uma boa alternativa terapêutica para avaliar sua atividade biológica em alguns modelos de câncer.

Palavras-chave: câncer, muscarínico, antagonistas, angiogênese.

1. Introduction

Several statistical data indicate that cancer has increased dramatically in recent years, resulting in a higher mortality rate worldwide (Chhikara and Parang, 2022; Siegel et al., 2023; Shang et al., 2024). Some risk factors involved in cancer development have been detected, such as demographics (Costa et al., 2024), hormones (Liu et al., 2023), smoking (Anbarasu and Anbarasu, 2023), heredity (Liu et al., 2023), and some parameters involved and lifestyle (Kwon et al., 2023). In addition, different reports indicate that some cancers are associated with the cholinergic system through muscarinic receptor activation (Khon et al., 1996; Wan et al., 2000; Shah et al., 2009; Zhao et al., 2015; Yu et al., 2017; Tolaymat et al., 2019; Liu et al., 2019;); for example, a study showed that muscarinic receptors (M_1 and M_2) are expressed in two murine mammary adenocarcinoma cell lines, LM2 and LM3, in BALB/c mice (Español and sales, 2004).

Another study indicates that the muscarinic-3 receptor (M_3) contributes to breast cancer tumorigenesis through angiogenesis regulation using an *in vitro* model (Chen et al., 2020). Besides, a report displayed that the administration of carbachol (muscarinic agonist) with paclitaxel (promoter of microtubule assembly from tubulin dimers) can inhibit the growth of human MCF-7 tumor cells (Salem et al., 2020). Other data indicate that M_1 and M_3 expressed in LMM3 mammary tumor cells may positively regulate proliferation and angiogenesis required for cancer development (Rimmaudo et al., 2005).

Besides, a report suggests that muscarinic cholinergic receptor activation induces MCF-7 cell proliferation and angiogenesis by increasing the biological activity of nitric oxide synthase (Fiszman et al., 2007). All this data suggests that muscarinic receptor activation can stimulate tumor progression; however, there are other types of biomolecules that can regulate the growth of cancer through angiogenesis activation. It is noteworthy that the angiogenesis process is regulated by several biomolecules, such as vascular endothelial growth factor (VEGF) through the VEGF-R1, VEGF-R2, and VEGF-R3 activation, which can be expressed in several cancers (Feurino et al., 2007; Ahmad et al., 2022; Eguchi et al., 2022; Sergolu et al., 2022). For example, a study showed that both VEGFR-1 and VEGFR-2 are expressed in bladder squamous cell carcinoma cells using the Western immunoblotting method (Klasa-Mazurkiewiez et al., 2011). Another study suggests that VEGFR-2 and VEGFR-3 are expressed in ovarian cancer patients using the Western blot technique (Kopparapu et al., 2013).

Besides, a report showed that VEGFR-1 can regulate the biological activity of epidermal growth factor receptor to increase colon cancer cell growth using the Western blot method (Naganu et al., 2019). All this data suggests that several cancer growths can involve muscarinic and VEGFR receptor activation. This phenomenon could be supported by studies that indicate that carbachol increased the constitutive expression of VEGF-A in tumor cells, and this effect was reverted by the muscarinic antagonist atropine (Lombardi et al., 2013). Besides, a study showed that blockade of VEGFR-1 and VEGFR-2 with bevacizumab enhances sensitivity to paclitaxel in TUBB3-expressing gastric cancer cells (Hwang et al., 2013).

Analyzing this data, this study aimed to determine the possible interaction of some muscarinic antagonists with VEGFR-1, VEGFR-2, and VEGFR-3 receptors using a theoretical model.

2. Materials and Methods

Muscarinic antagonists such as atropine [non-selective antagonist] (Nakajima et al., 2023), pirenzepine (Myslivcek and Farar, 2024), azapropone (Carroll et al., 1991), darifenacin [M_2 antagonist] (Hoshi and Ehlert, 2024), dicyclomine (Ali et al., 2018), AFDX-386 (Holzgrabe and Heller, 2003), ML381 (Gentry et al., 2014), PD 102807 (Tompkins et al.,

2023), telenzepine (Movalhedi et al., 2024), and zamifenacin (Cossy et al., 1997) were used to evaluate their possible interaction with vascular endothelial growth factor receptors (VEGF-R1, R_2, and R_3) using 2oh4, 3hng, and 4bsj proteins as theoretical tools.

2.1. Protein-Ligand

The theoretical interaction of muscarinic antagonists with VEGF-R1, R_2, and R_3 was determined using DockingServer (Bikadi & Hazai, 2009). In this method the MMFF94 force field (Halgren, 1998) was used for energy minimization of the ligand molecule (muscarinic antagonist) using DockingServer. Gasteiger partial charges were added to the ligand atoms. Besides, non-polar hydrogen atoms were merged, and rotatable bonds were defined. Other docking calculations were carried out on some protein models registered in the databank, such as 2oh4 (https://doi.org/10.2210/pdb2OH4/pdb), 3hng (https://doi.org/10.2210/pdb3HNG/pdb), and 4hbsj (https://doi.org/10.2210/pdb4BSJ/pdb). It is noteworthy that essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of AutoDock tools (Morris et al., 1998). Affinity (grid) maps of 20×20×20 Å grid points and 0.375 Å spacing were generated using the Autogrid program [39]. In addition, AutoDock parameter set and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively. It is noteworthy that docking simulations were performed using the Lamarckian genetic algorithm (Solis and Roger, 1981). Initial position, orientation, and torsions of the ligand molecules were set randomly. Each docking experiment was derived from 2 different runs that were set to terminate after a maximum of 250000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å, and quaternion and torsion steps of 5 were applied.

2.2. Pharmacokinetics parameter

Some pharmacokinetic factors for muscarinic antagonist were determined using the SwissAdme software (Figueroa-Valverde et al., 2012).

2.3. Toxicology analysis

Toxicology evaluation for muscarinic antagonist were determined using the Gussar software (Figueroa-Valverde et al., 2022).

3. Results

Theoretical data on the interaction of muscarinic antagonists with vascular endothelial growth factor receptors (VEGF-R1, R_2, and R_3) using 2ho4, 3hng, and 4sbj proteins as theoretical tools in the DockingServer program are shown as follows:

3.1. Ligand-protein complex

Table 1 and (Figure 1 shows the interaction of some muscarinic antagonist drugs with the 2oh4 protein surface. The results suggest that muscarinic antagonists could interact with the different number of aminoacid residues involved in 2oh4 protein surface compared with cabozantinib and cediranib. Other results shown indicate differences in the aminoacid residues involved in the coupling of muscarinic antagonist with 3hng protein. Finally, the results for the coupling of muscarinic antagonists with the 4bsj protein surface are displayed in Table 1.

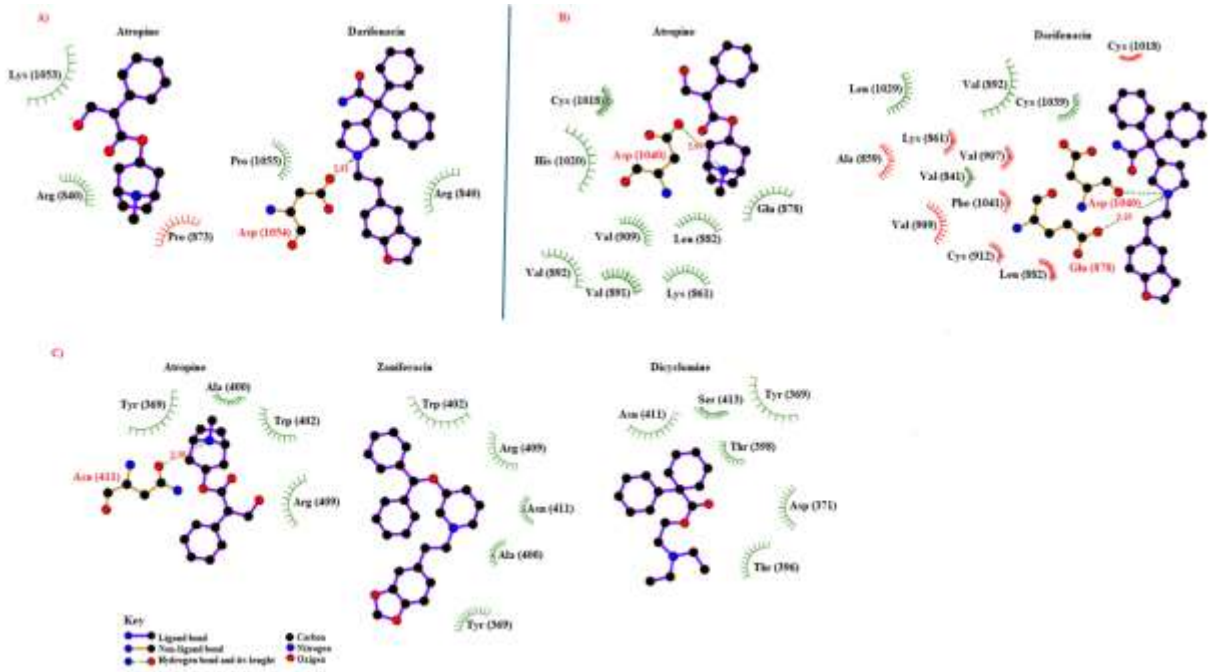

Figure 1. Coupling of atropine and dariferacin with 2oh4 (A) and 3hng (B) protein surfaces. Besides, The schem C showed the interaction of atropine and zaniferacion with 4bsj protein surface. Visualizated with DockingServer program.

Table 1. Interaction of muscarinic antagonist with aminoacid residues involved in the 2oh4, 3hng, and 4bsj proteins surface

	2oh4	3hng	4bsj
Cabozantinib*	Arg_{840}; Arg_{1049}; Ile_{1051}; Lys_{1053}; Asp_{1054}	Val_{841}; Ala_{859}; Lys_{861}; Glu_{878}; Ile_{881}; Leu_{882}; Val_{892}; Val_{907}; Val_{909}; Cys_{1018}; His_{1020}; Leu_{1029}; Ile_{1038}; Cys_{1039}; Asp_{1040}; Phe_{1041}	
Cediranib*	Arg_{840}; $Lys_{869;}$ Arg_{1049}; Lys_{1053}; Asp_{1054}; Pro_{1055}		Tyr_{369}; Ala_{400}; Trp_{402}; Arg_{409}; Asn_{411}
Axitinib		Val_{841}; Glu_{878}; Ile_{881}; Leu_{882}; Val_{891}; Val_{892}; Leu_{1013}; Cys_{1018}; His_{1020}; Leu_{1029}; Ile_{1038}; Cys_{1039}; Asp_{1040}; Phe_{1041}	Ala_{400}; Leu_{401}; Trp_{402}; Arg_{409}; Arg_{410}; Asn_{411}
Regorafenib		Val_{841}; Ala_{859}; Lys_{861}; Glu_{878}; Leu_{882}; Ile_{885}; Ile_{881}; Val_{892}; Val_{907}; Val_{909}; Cys_{912}; Leu_{1013}; Cys_{1018}; Ile_{1019}; His_{1020}; Leu_{1029}; Asp_{1040}; Phe_{1041}	
Pazopanib		Leu_{833}; Glu_{878}; Leu_{882}; Val_{892}; Val_{909}; Tyr_{911}; Cys_{912}; His_{1020}; Leu_{1029}; Cys_{1039}; Asp_{1040}; Phe_{1041}	
atropine	$Pro_{837;}$ Arg_{840}; Lys_{1053}	$Lys_{861;}$ Glu_{878}; Leu_{882}; Val_{891}; Val_{892}; $Val_{909;}$ $Cys_{1018;}$ $His_{1020;}$ Asp_{1040}	Tyr_{369}; Ala_{400}; $Trp_{402;}$ $Arg_{409;}$ Asn_{411}
azaprophen	Arg_{840}; $Lys_{1053;}$ Asp_{1054}	Thr_{877}; Glu_{878}; $Ile_{881;}$ Leu_{882}; Val_{891}; $Cys_{1018;}$; $His_{1020;}$ Asp_{1040}	Tyr_{369}; Ala_{400}; $Trp_{402;}$ $Arg_{409;}$ Asn_{411}
Darifenacin	Arg_{840}; Asp_{1054}; Pro_{1055}	Val_{841}; Ala_{859}; $Lys_{861;}$ Glu_{878}; Leu_{882}; Val_{892}; Val_{907}; Val_{909}; $Cys_{912;}$ $Cys_{1018;}$ $Leu_{1029;}$ $Cys_{1039;}$ $Asp_{1040;}$ Phe_{1041}	Ala_{400}; $Trp_{402;}$ $Ser_{404;}$ $Arg_{409;}$ Asn_{411}
ML381	Arg_{840}; Ala_{842}; $Lys_{869;}$ Arg_{1030}; Arg_{1049}; $Asp_{1050;}$ $Lys_{1053;}$ Asp_{1062}	Val_{841}; Ala_{859}; $Lys_{861;}$ Glu_{878}; $Ile_{881;}$ $Leu_{882;}$ Val_{892}; $Val_{909;}$ $Leu_{1029;}$ $Asp_{1040;}$ Phe_{1041}	Tyr_{369}; $Thr_{398;}$ Ala_{400}; $Trp_{402;}$ $Arg_{409;}$ Asn_{411}
PD 102807	Arg_{840}; Ala_{842}; $Lys_{869;}$ Arg_{1049}; $Lys_{1053;}$ Asp_{1054}	Glu_{878}; $Ile_{881;}$ $Cys_{1018;}$ $His_{1020;}$ $Arg_{1021;}$ $Asp_{1022;}$ $Asp_{1040;}$ $Arg_{1045;}$ Tyr_{1053}	Tyr_{369}; Ala_{400}; $Trp_{402;}$ $Ser_{404;}$ $Arg_{409;}$ Asn_{411}
Pirenzepine	Arg_{840}; Asp_{1054};	$Asp_{807;}$ Thr_{877}; Glu_{878}; $Ile_{881;}$ $His_{1020;}$ $Arg_{1021;}$ Asp_{1040}	Tyr_{369}; Ala_{400}; $Trp_{402;}$ $Arg_{409;}$ Asn_{411}
Telenzepine	Arg_{840}; $Lys_{869;}$ Asp_{1054}	Glu_{878}; $Ile_{881;}$ $Leu_{882;}$ $Ile_{885;}$ Val_{891}; Val_{892}; Leu_{1013}; $Ile_{1038;}$ $Cys_{1039;}$ Asp_{1040}	Tyr_{369}; Ala_{400}; $Trp_{402;}$ $Arg_{409;}$ Asn_{411}
AFDX-386	$Pro_{837;}$ Arg_{840}; Arg_{1049}; Lys_{1053}	Val_{841}; $Lys_{861;}$ Glu_{878}; Leu_{882}; Val_{892}; Val_{909}; Leu_{1013}; $Cys_{1018;}$ $His_{1020;}$ $Cys_{1039;}$ Asp_{1040}	$Gln_{367;}$ Tyr_{369}; $Ala_{374;}$ Ala_{400}; $Trp_{402;}$ $Arg_{409;}$ Asn_{411}
Zamifenacin	Arg_{840}; $Lys_{869;}$ Arg_{1049}; $Lys_{1053;}$ $Asp_{1054;}$ $Pro_{1055;}$	Val_{841}; $Lys_{861;}$ Glu_{878}; $Ile_{881;}$ $Leu_{882;}$ $Ile_{885;}$ Val_{891}; Val_{892}; $Val_{909;}$ Leu_{1013}; $Cys_{1018;}$ $His_{1020;}$ $Leu_{1029;}$ $Cys_{1039;}$ $Asp_{1040;}$ Phe_{1041}	Tyr_{369}; Ala_{400}; $Trp_{402;}$ $Arg_{409;}$ Asn_{411}
Dicyclomine	Arg_{840}; $Lys_{1053;}$ Asp_{1054}	$Asp_{807;}$ Thr_{877}; Glu_{878}; $Ile_{881;}$ $Leu_{882;}$ $Ile_{885;}$ Val_{891}; Leu_{1013}; $Cys_{1018;}$ $His_{1020;}$ $Ile_{1038;}$ Asp_{1040}	Tyr_{369} ; Asp_{371}; Thr_{396}; Thr_{98}; Asn_{411}; Ser_{413}

3.2 Thermodynamic parameters

Table 2 displays differences in values of energies involved in the possible interaction of muscarinic antagonists with the 2oh4 protein surface. Other data showed that the inhibition constant (Ki) was lower for atropine and defernacin compared with cabozantinib and cediranib.

Table 2. Thermodynamic parameters involved in the interaction of muscarinic antagonists with 2oh4 protein surface.

Compound	**A**	**B**	**C**	**D**	**E**	**F**
Cabozantinib	-5.15	168.22	-5.81	-0.18	-5.99	671.90
Cediranib	-4.53	474.23	-4.75	-0.39	-5.14	615.74
Atropine	-3.37	3.37	-4.34	+0.27	-4.07	545.721
Azaprophen	-4.48	522.19	-5.52	-0.61	-6.12	574.579
Darifenacin	-3.85	1.50	-3.78	-1.09	-4.87	544.716
ML381	-4.83	289.47	-6.50	-0.38	-6.88	767.532
PD 102807	-4.87	268.96	-5.62	-0.17	-5.80	627.121
Pirenzepine	-4.82	295.28	-4.62	-0.17	-4.79	527.046
Telenzepine	-4.29	711.50	-4.10	-0.37	-4.47	537.589
AFDX-386	-6.08	34.67	-6.89	-0.07	-6.96	669.312
Zamifenacin	-4.10	979.80	-5.53	+0.44	-5.09	649.197

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.

Other data suggest that the interaction of sorafenib with 3hng protein surface was lower compared with Ki values for muscarinic antagonists (Table 3).

Table 3. Thermodynamic parameters involved in the coupling of muscarinic antagonists with 3hng protein surface.

Compound	**A**	**B**	**C**	**D**	**E**	**F**
Cabozantinib	-7.70	2.28	-8.77	-0.18	-8.95	1000.65
Pazopanib	-8.76	380.77	-10.15	-0.11	-10.76	999.38
Regorafenib	−5.05	198.17	−6.84	−0.09	−6.93	1004.77
Sorafenib	−7.03	7.03	−8.19	−0.23	−8.42	922.58
Atropine	-6.92	8.46	-7.66	-0.63	-8.29	718.332
Azaprophen	-6.40	20.39	-7.75	-0.05	-7.79	766.695
Darifenacin	-6.82	9.99	-9.46	-0.19	-9.64	971.668
ML381	-8.37	727.03	-10.16	-0.13	-10.29	963.703
PD 102807	-5.33	124.28	-6.44	-0.11	-6.55	821.233
Pirenzepine	-6.09	34.15	-5.67	-0.40	-6.07	760.263
Telenzepine	-6.10	33.93	-6.07	-0.55	-6.62	761.555
AFDX-386	-8.99	259.31	-9.68	-0.10	-9.78	834.62
Zamifenacin	-5.08	190.24	-8.79	+0.06	-8.73	950.538

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.

Finally, other results (Table 4) suggest that Ki for atropine, zamiferacin, and dicyclomine was lower compared with axitinib and cediranib.

Table 4. Thermodynamic parameters con 4bsj

Compound	**A**	**B**	**C**	**D**	**E**	**F**
Axitinib	-6.96	7.87	-7.74	0.00	-7.74	629.46
Cediranib	-4.92	248.37	-4.71	0.11	-4.60	475.52
Atropine	-3.88	1.44	-4.34	+0.25	-4.09	506.425
Azaprophen	-4.23	788.21	-5.31	+0.21	-5.10	521.597
Darifenacin	-5.13	173.21	-7.50	+0.35	-7.16	665.966
ML381	-4.67	375.60	-6.03	+0.01	-6.02	626.077
PD 102807	-4.93	244.51	-5.96	-0.06	-6.02	605.979
Pirenzepine	-5.55	84.82	-5.19	+0.10	-5.08	465.984
Telenzepine	-5.57	82.78	-5.58	+0.27	-5.32	529.712
AFDX-386	-5.14	171.83	-5.82	-0.05	-5.88	530.625
Zamifenacin	-3.60	2.28	-4.83	+0.14	-4.69	507.566
Dicyclomine	-4.00	1.17	-4.21	-0.14	-4.35	497.087

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.

3.3. Pharmacokinetic evaluation

The results (Table 5) indicate that the metabolism of atropine, ML381, zamiferacin, and dicyclomine involves different CYPs (P450 family).

Table 5 Thermodynamic parameters values for muscarinic antagonists.

Parameter	**Atropine**	**ML381**	**Zamifenacin**	**Dicyclomine**
GI absorption	High	High	High	High
BBB permeant	Yes	No	Yes	Yes
P-gp substrate	No	No	Yes	No
CYP1A2 inhibitor	No	Yes	No	Yes
CYP2C19 inhibitor	No	Yes	No	No
CYP2C9 inhibitor	No	Yes	Yes	No
CYP2D6 inhibitor	Yes	No	Yes	Yes
CYP3A4 inhibitor	No	Yes	Yes	No
Consensus Log $P_{o/w}$	2.06	2.56	4.79	4.42

3.4. Toxicity analysis

The results (Table 6) suggest that different doses of atropine, ML381, zamiferacin, and dicyclomine are required to produce some degree of toxicity.

Table 6. Thermodynamic parameters con 4bsj

Drug	Rat IP LD50 (mg/kg)	Rat IV LD50 (mg/kg)	Rat Oral LD50 (mg/kg)	Rat SC LD50 (mg/kg)
Atropine	105,400	35,010	1398,000	273,200
ML381	629.700	47.570	1358.000	666.900
Zamifenacin	202.400	13.230	842.900	229.800
Dicyclomine	229.700	42.82	1403.000	543.400

IP - Intraperitoneal route of administration
IV - Intravenous route of administration
Oral - Oral route of administration
SC - Subcutaneous route of administration

4. Discussion

There are some studies in the literature indicating that cancer development is associated with the angiogenesis development. It is important to mention that different reports suggest that angiogenesis may be conditioned by the synthesis/release of several biomolecules such as muscarinic receptors (M1-M5) and vascular endothelial growth factor receptors (VEGF-R1, R2, and R3). In this way, some muscarinic antagonists have been used to evaluate their biological activity on VEGF-R1, R2, and R3; however, their interaction with these biomolecules is confusing. Analyzing this data, the aim of this study was to evaluate the coupling of some muscarinic receptor antagonists with VEGF-R1, R2, and R3 using a theoretical model as followed:

4.1 Protein Interaction

Several theoretical methods have been used to determine the interaction of some drugs with VEGF-R1, R2, and R3 receptors; for example, a study showed that isoliquiritigenin could modulate the VEGF/VEGF-R2 signaling pathway using the Discovery Studio program [Wang et al., 2013]. Other data indicate that some phthalazine derivatives may interact with VEGFR-2 with anticancer activity using Molsoft software (EL-Helby et al., 2017). Besides, a study showed that some pyrazole derivatives interact with VEGFR-2, which could act as a potent anti-breast cancer using MOE 2008 software (Pauling and Petcher, 1970). Analyzing these data, the aim of this study was to determine the possible interaction of some muscarinic antagonists, such as atropine (non-selective muscarinic receptors). [48], ML381 [M_5-muscarinic receptor antagonist] (Gentry et al., 2014), af-dx 386 [M_2-muscarinic receptor antagonist] (hazan et al., 2024), azaprophen [M_2-muscarinic receptor blocker] (Carrol et al., 1991), darifenacin M_3-muscarinic receptor antagonist] (Haab et al., 2004), dicyclomine [M_1-muscarinic receptor blocker] (Ali et al., 2018), PD 102807 [M_4-muscarinic receptor antagonist] (Olianas and Onali, 1999), pirenzepine [M_1-muscarinic receptor blocker] (Eskazan et al., 1999), telenzepine [M_1-muscarinic receptor antagonist] (Cazzola et al., 1994), Zamifenacin [M_3-muscarinic antagonist] (Houghton et al., 1997), cyclohexylamine derivative (Li et al., 2020) using some drugs such as cabozantinib [VEGFR-2 inhibitor] (Yakes et al., 2011), cediranib [VEGFR-1 and 2 blocker] (Brave et al., 2011), axinib [VEGFR1-3 inhibitor)] (Rossler et al., 2011), pazonib [VEGFR-2/3 blocker] (Sloan et al., 2008), and regorafenib [VEGFR1-3 inhibitor] (Wilhelm et al., 2011) as theoretical tools in DockingServer program. The results showed that there are differences in the number of aminoacid residues involved in the interaction of muscarinic antagonists with 2oh4, 3hng, and 4bsj protein surfaces. Besides, the inhibition constant (Ki) involved in the interaction of muscarinic antagonists with the 2ho4 protein surface for atropine and darifenacin drugs was lower compared with cabozantinib and cediranib. This phenomenon could be due to the interaction of different aminoacid residues; for example, the atropine may bind to Arg_{840} and Lys_{1053} via polar bonds and Pro_{837} through hydrophobic bonds.

Other data suggested that interaction of for cabozanitib and sorafenib with 3hng protein surface was lower compared with Ki values for muscarinic antagonist. However, the Ki for atropine and darifenacin was lower compared with pazopanib and regorafenib drugs. These results could be due to the type of interaction for atropine with some aminoacid residues such as Asp_{1040} via hydrogen bond, Glu_{878} and Asp_{1040} through polar bond, and Leu_{882}, Val_{892}, Val_{909}, Cys_{1018} and His_{1020}. Besides, the coupling of darifenacin with 3hng protein surface involves different aminoacid residues such as Glu_{878} and Asp_{1040} via hydrogen bonds; Lys_{861} and Glu_{878} polar bonds; Val_{841},

Ala_{859}, Val_{892}, Val_{907}, Val_{909}, Cys_{912}, Leu_{1029} and Cys_{1039} through hydrophobic bonds. Finally, other data indicate that the Ki value involved in the interaction of atropine, zaniferacin, and dicyclomine with the 4bsj protein surface was lower compared with axitinib and cedirinib. In this way, the coupling for atropine with some aminoacid residues such as Asn_{411} was via hydrogen bonds, with Tyr_{369} and Asn_{411} through polar bonds, and with Ala_{400}, Trp_{402} via hydrophobic bonds. All this data suggests that muscarinic receptor antagonists such as atropine, darifenacin, zaniferacin, and dicyclomine could condition the biological activity of angiogenesis through their interaction with VEGF receptors, resulting in possible changes in cancer cell growth.

4.2. Pharmacokinetic and toxicological analysis

For several years, different drugs to treat cancers have been developed; however, some of these drugs can produce several secondary effects, which result in a decreased quality of life for patients with cancer. In the search for new therapeutic alternatives, some computational and experimental models have been used, which involve the pharmacokinetic and toxicological analysis of new drugs. For example, several methods have been used to predict some pharmacokinetic parameters, such as PKQuest (Levitt, 2012), PharmPK (Bourne, 1997), and SwissADME (Sicak, 1997), and others. In this investigation, some pharmacokinetic parameters involved in muscarinic antagonists such as atropine, ML-381, zaniferacin, and dicyclomine were determined using the SwissADME program. The results suggest that metabolism of muscarinic antagonists could involve different Cyps (P_{450} family). Besides, the absorption of atropine, darifenacin, zaniferacin, and dicyclomine was greater; however, the level of lipophilicity is higher for zaniferacin and dicyclomine compared with atropine and darifenacin; this phenomenon could condition the volume of distribution of these drugs.

On the other hand, it is noteworthy that some methods have been used to predict the toxicity degree of new drugs (Poland, 1973). Analyzing these data, the aim of this study, the possible toxicity produced by atropine, ML-381, zaniferacin, and dicyclomine, was determined using the GUSAR program [(Figueroa-Valverde et al., 2022). The results (Table 8) indicate that toxicity could depend on the dose administered for atropine, ML-381, zaniferacin, and dicyclomine through different routes of administration. For example, ML381 drugs require a higher dose via intravenous, intraperitoneal, and subcutaneous routes to produce some degree of toxicity compared with atropine, zaniferacin, and dicyclomine.

5. Conclusions

In conclusion, theoretical results indicate that the muscarinic antagonist atropine, ML-381, zaniferacin, and dicyclomine could act as VEGF receptor inhibitors, resulting in changes in the biological activity of angiogenesis, and this phenomenon could be translated as a decrease in cancer cell growth. Therefore, these muscarinic antagonists could be a good therapeutic alternative to evaluate their biological activity in some cancer models.

6. Authors' Contributions

Substantial contribution to research design: *Lauro Figueroa-Valverde* and *Marcela Rosas-Nexticapa*; Acquisition, analysis and interpretation of data: *Lauro Figueroa-Valverde*, *Rosas-Nexticapa*, *Magdalena Alvarez-Ramirez*, *Maria Virginia Mateu-Armad* and *Regina Cauich-Carrillo*. Approval of the submitted and final versions: all authors.

7. Conflicts of Interest

No conflicts of interest.

8. Ethics Approval

Does not apply.

9. References

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