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In Silico Interaction of Select Cardiovascular Drugs with the Developmental Signal Pathway Pax3

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Abstract:

Introduction: Paired Box 3 Pax3 is a pivotal protein in embryogenesis. This study computationally examines the interaction between twenty cardiovascular drugs and Pax3. Many of these drugs can cross the placenta, suggesting their potential influence on embryonic safety during pregnancy. This investigation is crucial due to Pax3's central role in embryogenesis.

Methods: The Zinc 15 database was utilized to extract the 3D chemical structures of the selected drug molecules, while the Pax3 protein was obtained from the Protein Data Bank. Subsequently, the structures of both the molecules and the protein were prepared, and the docking process was conducted using Discovery Studio and the Virtual Screening Tool Python Prescription.

Results: The majority of the drugs exhibited a propensity for protein binding, with affinity values between them and the Pax3 protein ranging from (-8.3) to (-4.8). Only six of them displayed weak affinities below (-6). The findings unveiled that all of these drugs engaged in varied bonding with amino acids situated within the protein's active site. Furthermore, most of them formed both conventional and unconventional hydrogen bonds with the protein's amino acids.

Conclusion: These findings highlight intricate interactions between cardiovascular drugs and Pax3 protein. Notably, fetal health in pregnant women using these drugs during pregnancy appears inversely related to their interaction with Pax3. This correlation accounts for both the strength and precision of binding. Consequently, among the studied drugs, Losartan appears safer for fetal development compared to Spironolactone and Ouabain.

Keywords: Pax3, cardiovascular drugs, interaction, pregnancy, docking.

Introduction:

Physicians often prescribe cardiovascular drugs during pregnancy to address health concerns in both the mother and the developing fetus. Some mothers may also need to continue drug therapy after giving birth. Understanding the potential for many cardiovascular drugs to pass through the placenta is crucial, potentially exposing the developing embryo and fetus to their pharmacological effects. Such exposure could lead to adverse consequences for the fetus, including irreversible changes. Furthermore, a variety of medications

can be transferred into human breast milk, raising concerns about potential adverse effects on the breastfeeding infant. The complex physiological changes that occur during pregnancy, along with the drugs' pharmacokinetic properties, influence their impact on the nursing baby, as well as the inherent pharmacokinetic properties of each specific drug (Qasqas et al., 2004). Certainly, the flawless execution of any developmental program is truly remarkable. At the cellular level, all developmental processes are ultimately regulated by the collaborative functioning of different signal transduction pathways, including cellular protein signaling (Croce and McClay, 2009). These pathways coordinate intricate cellular behaviors during development, overseeing activities such as cell proliferation, the maintenance of stem cells, determining cell fate, as well as coordinating controlled cell movements and the establishment of tissue polarity (van Amerongen and Nusse, 2009). Any dysregulation or malfunction of cellular protein signaling pathways can lead to cellular abnormalities. These abnormalities may contribute to the development of various diseases and create congenital anomalies in the fetus (Nurden and Nurden, 2008; Gordon and Blobe; 2008; MacGrogan et al., 2018). Among those important cellular proteins is the Pax3 protein, which plays a crucial role in embryogenesis. It functions as a transcription factor, meaning it regulates the expression of specific genes by binding to their regulatory regions and influencing their activity. Pax3 exhibits early expression during embryonic development in specific spatial regions, including limb muscle, neural crest, and neural tube. In these domains, Pax3 plays a pivotal role in directing the differentiation of melanocyte stem cells, as well as in the processes of cardiogenesis and neurogenesis (Nakazaki et al., 2008; Goljanek-Whysall et al., 2011; Buckingham and Relaix, 2015). In addition to its role in embryogenesis, Pax3 mutations or abnormalities can lead to cellular abnormalities. Disruptions in Pax3 gene expression or function can result in developmental defects and congenital anomalies. These abnormalities may manifest as defects in the development of muscles, bones, and other tissues (Barber et al., 2002; Zalc et al., 2015; Qin et al., 2020; Palmer et al., 2021). The use of Cardiovascular medication has also been associated with adverse fetal events because several of these drugs have teratogenic potential (Davis et al., 2011; Ruys et al., 2014; Halpern et al., 2019). Handling cardiovascular disease during pregnancy presents significant challenges due to the distinct maternal physiology, marked by profound alterations in multiple organ systems. The presence of the fetus further complicates matters since both the cardiometabolic condition and its treatment may have adverse effects on the developing baby. Simultaneously, refraining from necessary treatment due to concerns about potential harm to the fetus can pose a risk of unfavorable outcomes for both the mother and the child (Ramlakhan et al., 2020). Overall, the goal remains to maintain the best possible health for both the mother and the baby during pregnancy, and this may involve a combination of medication, lifestyle changes, and close medical supervision. Due to there was no direct or established relationship between the Pax3 protein and cardiovascular drugs. In this study, we aim to determine the extent of the relationship between selected cardiovascular drugs and the transcription factor Pax3 computationally. The goal is to clarify the mechanism of the effect of these drugs on the fetus, given the protein's important and essential role in embryogenesis.

Material and methods:

Platform for molecular modelling: The docking process was performed via Discovery Studio (version 21.1.0.2) and Virtual Screening Tool Python Prescription (Version: PyRx-0.8).

Preparation of Ligand: The Zinc 15 database was used to extract out the 3D chemical structures of the selected molecules (https://zinc15.docking.org). The ligand was prepared, and its energy was minimized using the Virtual Screening Tool, Python Prescription (Version: PyRx-0.8).

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Preparation of protein structures and grid generation: PAX 3 protein (**NAKB:** 3CMY) under study was obtained from Protein Data Bank (https://www.rcsb.org). The protein structure was prepared and the water molecules and conformations were removed using protein preparation wizard Discovery Studio software. Further receptor grid boxes were generated using "Glide's Receptor Grid Generation" module at the active site after determining the active site based on a study Birrane et al. (2009).

Molecular docking: The binding strength between Pax3 protein and drug molecules used in the study was examined using the Tool Python Prescription (Version: PyRx-0.8). Then, docking between Pax3 protein and each of the studied drugs was carried out. The poses were ranked based on scoring values and the pose with the best scoring value was selected. The docking results were visualized using the Discovery Studio (version 21.1.0.2) for generating 2D and 3D pictures. Amino acids belonging to the active site that formed bonds with drug molecules were identified. In addition to hydrogen bonds.

Results:

The results of molecular docking for cardiovascular drugs suggest a pronounced tendency for most of these drugs to bind with the Pax3 protein. Affinity values between the twenty drugs and Pax3 protein ranged from (-8.3) to (-4.8), with 14 of them exhibiting affinities surpassing (-6). Out of these, only six displayed affinities below (-6) (refer to Table 1). Notably, Spironolactone, ouabain, and cortisone demonstrated the highest binding energy values, each boasting an affinity of (-8.3, -8.3, -8) respectively. Conversely, Nitroglycerin and Captopril exhibited the weakest binding energies, both recording an affinity of (-4.8) (refer to Table 1). A comprehensive analysis of the docking outcomes unveiled connections between all drugs and amino acids within the protein's active site, resulting in the formation of diverse bonds. Enalapril and Hydralazine, for instance, formed bonds with five amino acids at the active site, whereas the remaining drugs exhibited fewer connections (refer to Table 1, Figure 1). Furthermore, the results indicated that all drugs established conventional hydrogen bonds with amino acids in the Pax3 protein, with the exception of Losartan, which lacked this interaction. The count of conventional hydrogen bonds between drugs and protein amino acids varied from 1 to 4 (refer to Table 1, Figure 1). Regarding unconventional hydrogen bonds, such as carbon-hydrogen and Pi-Donor bonds, they were prevalent between most drugs and protein amino acids in varying quantities. However, these bonds were notably absent in six of the drugs studied: cortisone, Valsartan, Warfarin, Prasugrel, Losartan, and Misoprostol (refer to Table 1, Figure 1).

Table 1: The affinity values, interactions with amino acids in the active site, as well as both conventional and unconventional hydrogen bonds formed by the studied drugs with the Pax3 protein.

NO.	Drug name	Energy (Kcal/mol)	Active site interaction	Conventional H bond = length	others H bond = length
1.	Spironolactone	-8.3	ARG A: 44 ARG A: 3 THR A: 6 ARG A: 5	THR A: 6 = 3.88 ARG A: 5 = 5.69 ARG A: 55 = 3.87	ARG A: 44 = 4.37 ARG A: 3 = 4.78
2.	Ouabain	-8.3	ARG A: 3 THR A: 6	ARG A: 3 = 4.39 TRP A: 48 = 3.96	ARG A: 3 = 5.16

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			TRP A: 48 ASN A: 51	ASN A: 51 = 4.84	
3.	Cortisone	-8	ARG A: 5 TRP A: 48	ARG A: 5 = 4.51 TRP A: 48 = 5.23 ARG A: 55 = 6.16	-
4.	Ticagrelor (Brilinta)	-7	Val A:47 ARG A: 5 ARG A: 2 ARG A: 3	ARG A: 55 = 5.22 GLU A: 17 = 5.27 ARG A: 3 = 5.17	ARG A: 52 = 3.81 ARG A: 2 = 5.65
5.	Moexipril	-7	TRP A: 48 ARG A: 5 Val A: 47	ARG A: 55 = 4.80 ARG A: 5 = 4.89	ARG A: 52 = 4.63
6.	Valsartan	-7	ARG A: 5 Val A: 47	ARG A: 55 = 4.51	-
7.	Warfarin	-6.9	TRP A: 48 ARG A: 5 Val A: 47	TRP A: 48 = 5.53 ARG A: 5 = 5.13	-
8.	Prasugrel (Effient)	-6.8	TRP A: 48 ARG A: 44 THR A: 6	TRP A: 48 = 4.41 ARG A: 44 = 4.89 THR A: 6 = 3.66	-
9.	Benazepril (Lotensin)	-6.8	ARG A: 5 ASN A: 51 Val A: 47	ARG A: 5 = 5.75 ASN A: 51 = 5.22	ASN A: 51 = 4.81
10.	Vasopressin	-6.5	ARG A: 3 ASN A: 51 TRP A: 48 ARG A: 5	ARG A: 3 = 3.39; 3.22 SER A: 4 = 4.4.25 ASN A: 51 = 5.23 ARG A: 5 = 3.55; 2.98	TRP A: 48 = 5.17
11.	Enalapril	-6.4	ARG A: 44 ARG A: 3 Val A: 47	ASN A: 51 = 3.56 TRP A: 48 = 3.45 ARG A: 55 = 3.94	ARG A: 52 = 3.84

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			TRP A: 48 ASN A: 51		
12.	Losartan	-6.3	ARG A: 5 ARG A: 44 TRP A: 48 Val A: 47	-	-
13.	Clopidogrel (Plavix)	-6	ASN A: 51 TRP A: 48	TRP A: 48 = 5.11	ASN A: 51
14.	Amiodarone	-6	TRP A: 48 ARG A: 3	ARG A: 55 = 4.79	TRP A: 48 = 3.46 ARG A: 3 = 5.63
15.	Sotalol	-5.9	ARG A: 44 THR A: 6 Val A: 47	THR A: 6 = 3.00 ARG A: 55 = 4.41	ARG A: 44 = 4.59
16.	Misoprostol	-5.6	THR A: 6 Val A:47	THR A: 6 = 3.58	-
17.	Aspirin	-5.5	Val A: 47 ARG A: 44 THR A: 6	ARG A: 44 = 4.48 THR A: 6 = 3.45	ARG A: 3 = 4.86
18.	Hydralazine	-5.5	THR A: 6 ASN A: 51 Val A: 47 ARG A: 5 ARG A: 44	SER A: 4 = 5.14	THR A: 6 = 4.26 ASN A: 51 = 5.95
19.	Nitroglycerin	-4.8	TRP A: 48 ASN A: 51 ARG A: 44	TRP A: 48 = 5.20; 4.60 ASN A: 51 = 4.25	TRP A: 48 = 4.89
20.	Captopril	-4.8	THR A: 6 ARG A: 44 ARG A: 3	THR A: 6 = 3.49; 3.80; 3.88 ARG A: 44 = 5.03	ARG A: 3 = 4.43

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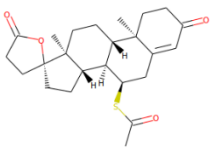
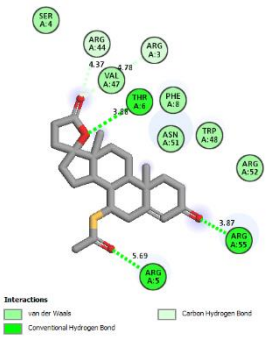
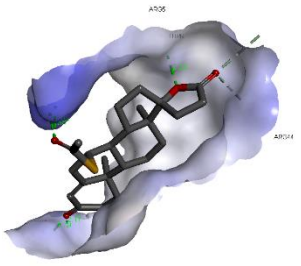
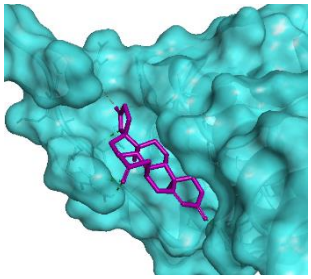
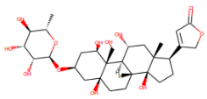
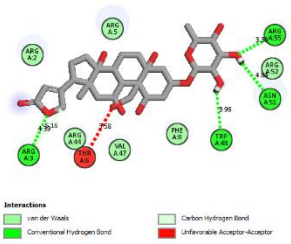
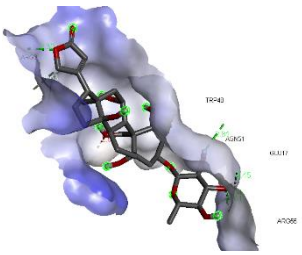
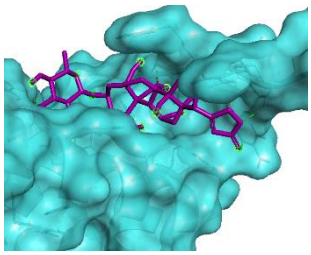
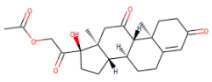
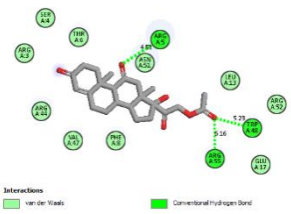
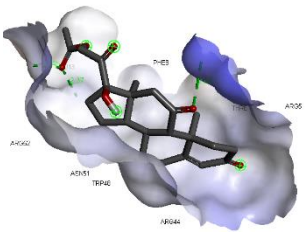
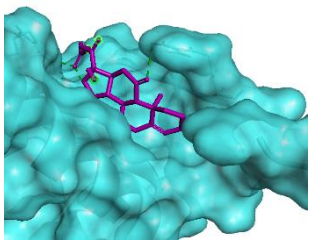
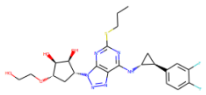
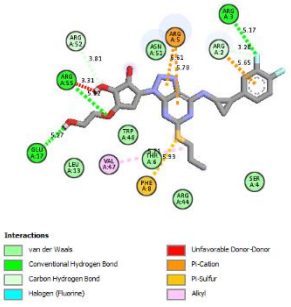
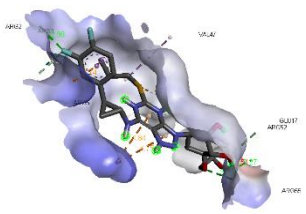
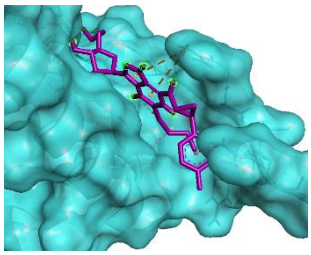
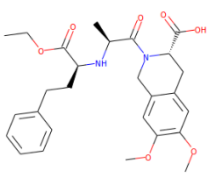
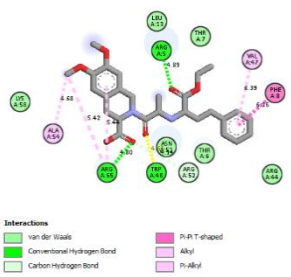
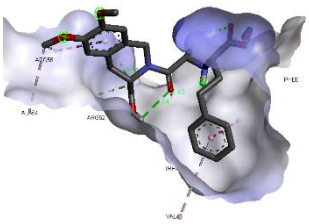
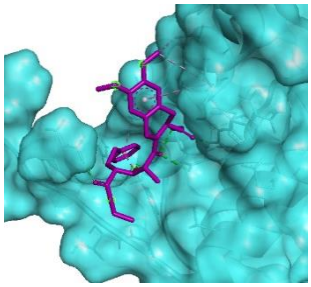
Table 2: The Drugs structure, 2D Interaction, 3D structure, and Interaction site of the studied drugs with the Pax3 protein.

	Drugs	2D Interaction	3D structure	Interaction site
1.	 Spironolactone			
2.	 Ouabain			
3.	 Cortisone			
4.	 Ticagrelor (Brilinta)			
5.	 Moexipril			

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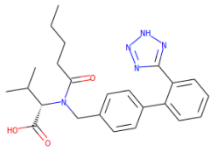
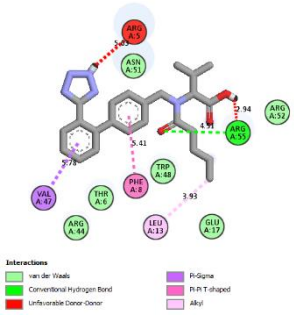
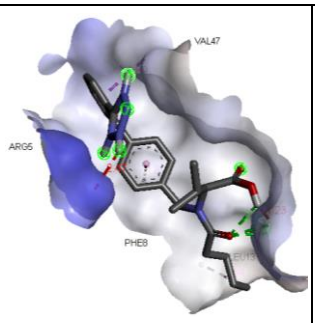
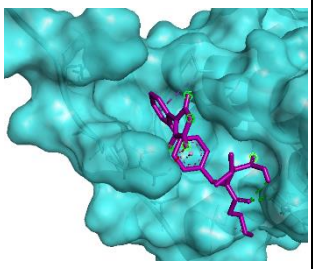
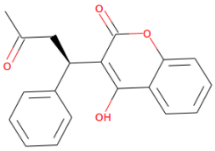
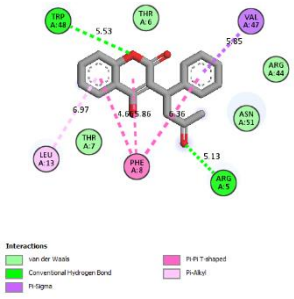
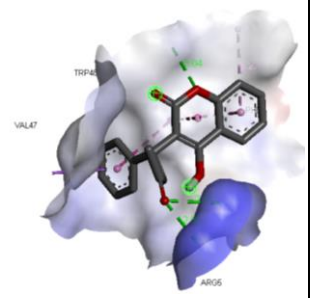
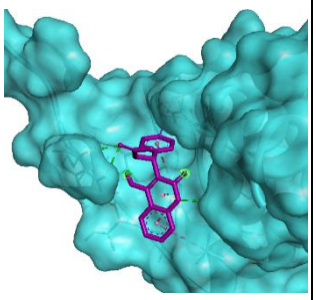
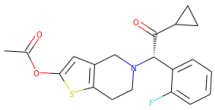
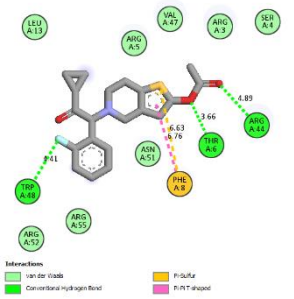
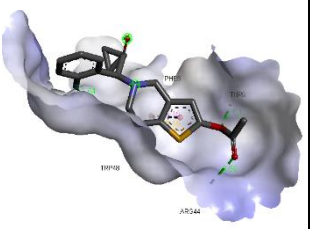
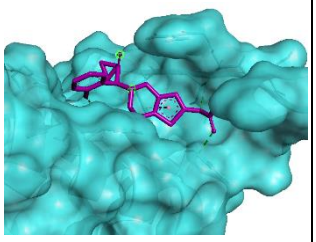
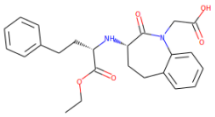
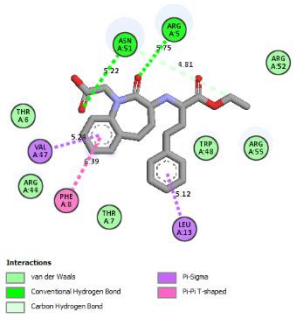
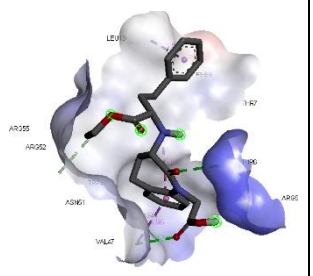
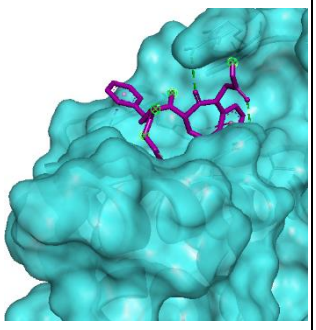
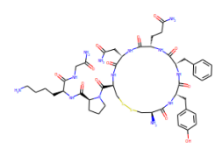
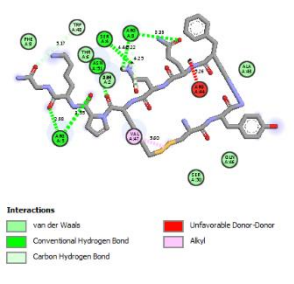
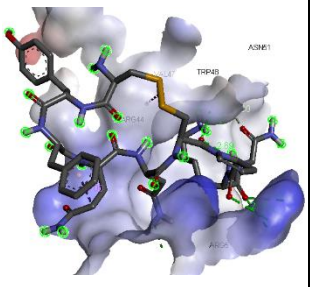
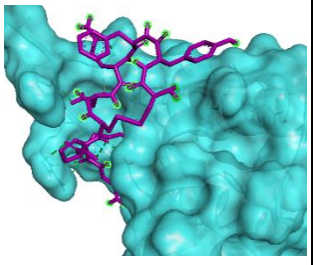
6.	 Valsartan			
7.	 Warfarin			
8.	 Prasugrel (Effient)			
9.	 Benazepril (Lotensin)			
10.	 Vasopressin			

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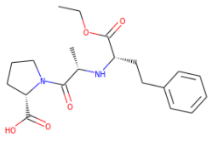
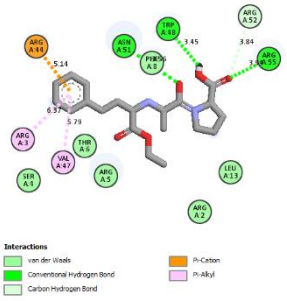
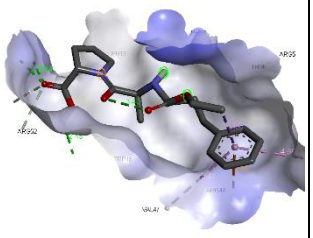
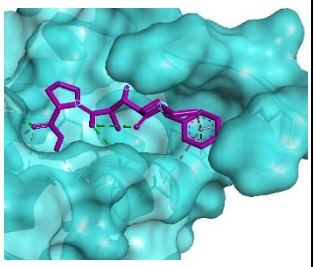
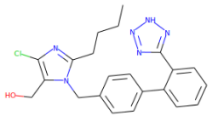
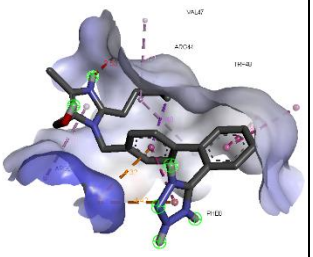
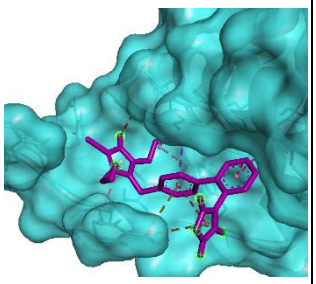
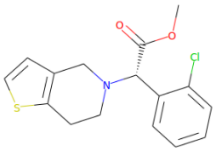
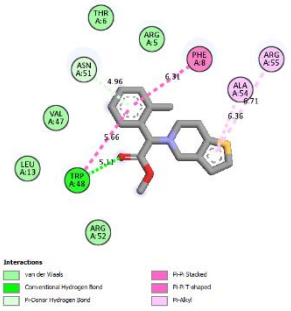
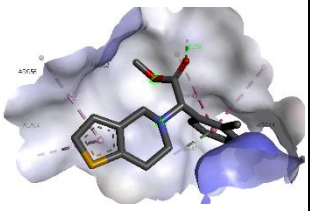
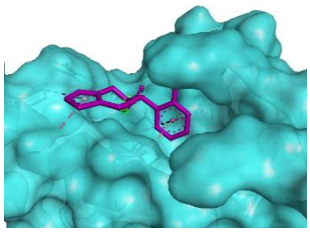
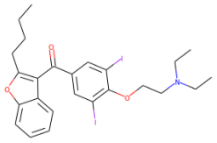
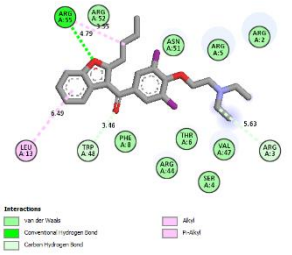
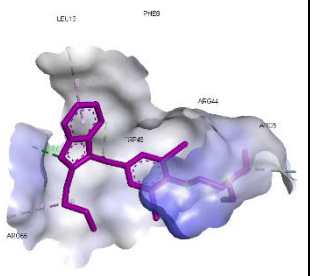
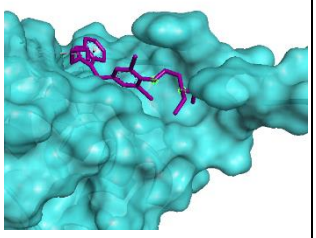
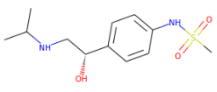
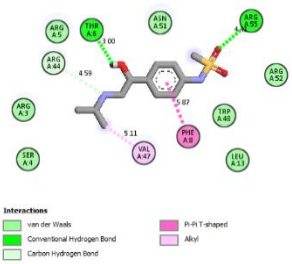
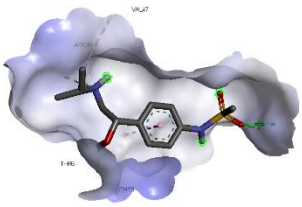
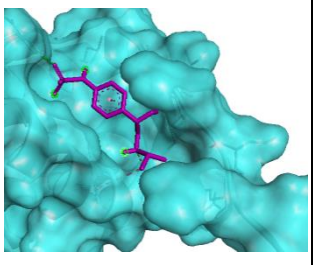
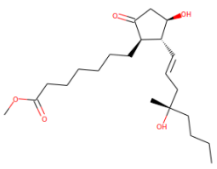
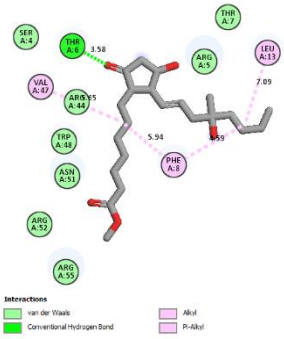
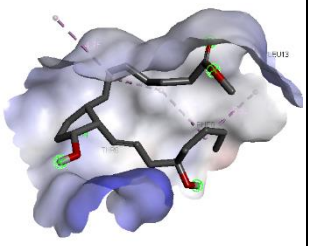
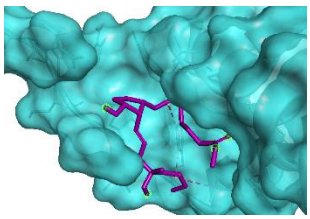
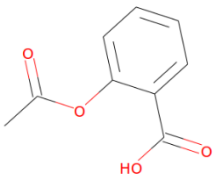
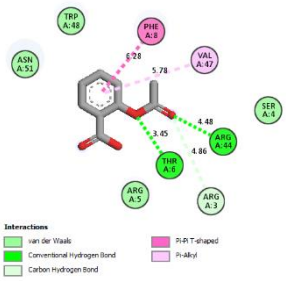
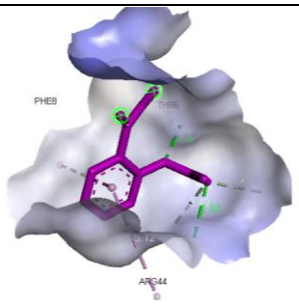
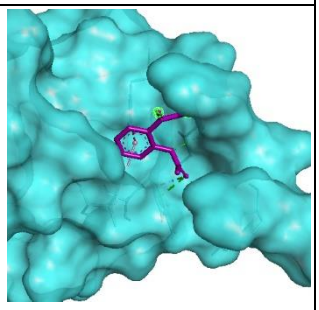
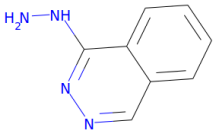
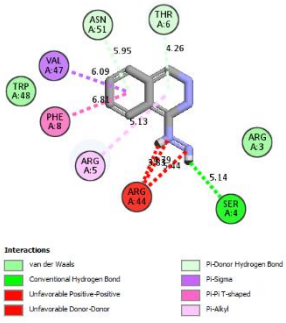
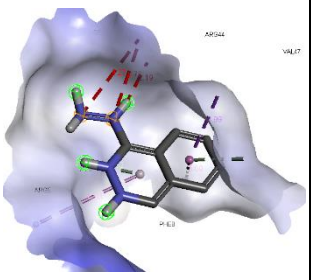
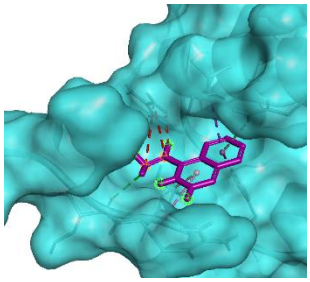
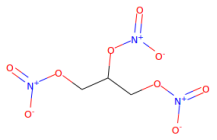
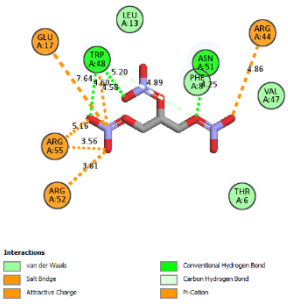
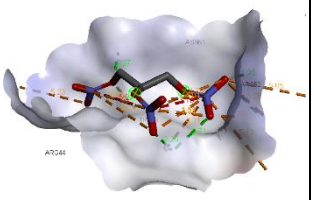
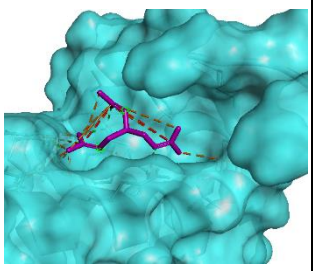
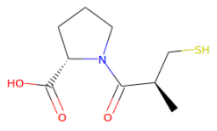
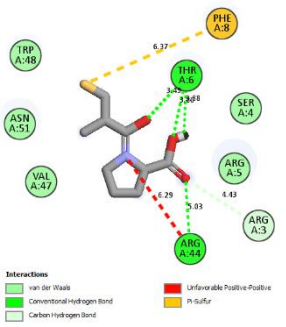
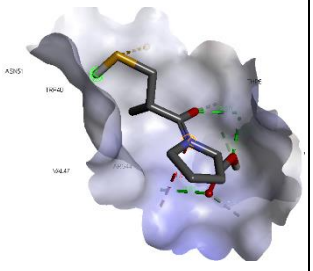
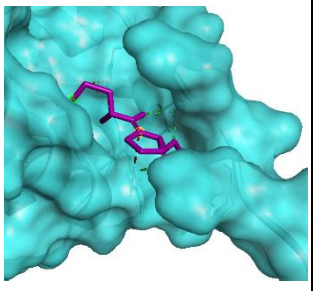
11.	 Enalapril			
12.	 Losartan			
13.	 Clopidogrel (Plavix)			
14.	 Amiodarone			
15.	 Sotalol			

16.	 Misoprostol			
17.	 Aspirin			
18.	 Hydralazine			
19.	 Nitroglycerin			
20.	 Captopril			

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Discussion:

The Pax3 protein, a transcription factor encoded by the Pax3 gene, is of utmost importance in embryogenesis. It holds a central role in the development of diverse embryonic tissues and structures (Nakazaki et al., 2008; Goljanek-Whysall et al., 2011; Buckingham and Relaix, 2015). Notably, mutations in the Pax3 gene are linked to congenital disorders and developmental irregularities. The significance of Pax3 in various developmental processes underscores its critical role in ensuring proper embryonic development (Barber et al., 2002; Zalc et al., 2015; Qin et al., 2020; Palmer et al., 2021).

The molecular docking results presented in this study demonstrate a pronounced tendency of various cardiovascular drugs to bind with the Pax3 protein. This finding raises concerns about the potential risks associated with taking these drugs during pregnancy. Given that mothers undergo substantial physiological changes during pregnancy, both the mother and the developing fetus become highly susceptible to the effects of medications (Forfar and Nelson, 1973; Soma-Pillay et al., 2016; Ramlakhan et al., 2020). The interaction between these drugs and the Pax3 protein could potentially hinder its function as a transcription factor, consequently disrupting critical embryonic developmental processes in which the protein plays a significant role. Our finding is consistent with some previous studies, which indicate that certain drugs can have adverse effects on the fetus if ingested by the mother during the early stages of pregnancy. Examples of such drugs include Warfarin (Rahul et al., 2022), aspirin (Garza-Galvan et al., 2023), and angiotensin-converting enzyme inhibitors (ACE inhibitors) such as Benazepril, Captopril, Enalapril, and Moexipril (Tomlinson and Feig et al., 2021). However, to the best of our knowledge, there has been no prior study investigating the relationship between these drugs and Pax3.

An escalation in the strength of protein binding for these drugs could suggest an elevated risk. Based on this premise, among the drugs under examination, spironolactone, ouabain, and cortisone appear to be the most hazardous. Conversely, it is not feasible to dismiss the potential risk associated with any of these drug types. Computational analysis of the examined drugs revealed the formation of diverse bonds with amino acids located within the protein's active site. This introduces the possibility of functional impairment in the protein, which could manifest as developmental disorders in the developing fetus. Notably, even among the medications reported to have a potential effect, their binding strengths varied; not all exhibited high affinity. For example, Moexipril, Warfarin, Benazepril, Enalapril, aspirin, and Captopril had affinity values of (-7, -6.9, -6.8, -6.4, -5.5, and -4.8) respectively, indicating different levels of interaction with the protein's active site.

Hydrogen bonds, both conventional and unconventional, are pivotal in binding interactions. A higher number of hydrogen bonds usually indicates stronger binding (Taylor et al., 2002). In our study, hydrogen bond presence predicts binding interactions between Pax3 and cardiovascular drugs. Strong conventional hydrogen bonds typically signify favorable interactions, while unconventional bonds contribute when conventional ones are limited (Taylor et al., 2002; Meng et al., 2011; Kaur et al., 2019). Most drugs in our study formed conventional hydrogen bonds with the protein, with around 14 drugs forming 2-4 bonds and five forming just one. Notably, Losartan didn't form any conventional hydrogen bonds. In terms of unconventional bonds, the majority of drugs interacted with the protein through 1-2 bonds, with only six drugs lacking this interaction.

These results highlight the necessity for additional studies and experiences to comprehend the effect of medications on the Pax3 protein and its influence on embryonic formation. This avenue could initiate innovation in the development of secure and effective drugs for addressing cardiovascular disorders during the pregnancy period. In brief, the outcomes of molecular docking

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demonstrate intriguing potential for innovation in the realm of treatment and cardiovascular disease research. They emphasize the significance of pursuing further research and studies to comprehend the effects and formulate secure and effective therapeutic strategies. The utilization of this technique can contribute to enhancing the design of forthcoming medications and directing development strategies.

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