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Estudo *in silico* de heterocíclicos tiofênicos frente a alvos de *Staphylococcus aureus*

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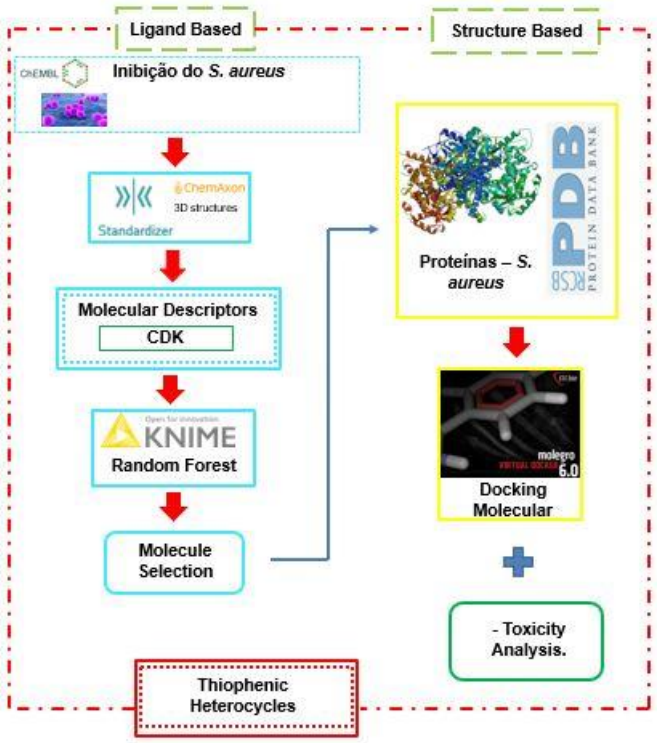
Graphical Abstract



Abstract

S. aureus é uma bactéria gram-positiva com formato esférico comumente encontrada na pele, sua infecção pode causar diversos problemas de saúde em diversas partes do corpo, sua infecção mais leve pode causar foliculite, coceira na pele, alopecia temporária e acumulo de cera seborreica na pele[1]. Além disso, as infecções podem comprometer além da pele, em seu estágio mais grave, outros órgãos como o fígado, o pâncreas, cérebro e coração. Devido à resistência bacteriana desse microrganismo, muitos pesquisadores buscam novos medicamentos antibacterianos para combater o S. aureus[2]. Este trabalho consiste em um estudo computacional de uma série de heterocíclicos tiofênicos frente a 2 alvos da bactéria S. aureus, com isso é possível aponta-los como protótipos a novos fármacos bactericida.

Materials and Methods

Initially, 30 molecules were submitted to a biological activity prediction model developed in the software KNIME Analytics Platform 3.8 [3] against the bacterium organism under study, using the classifier *Random Forest* and the predictor *Weka Predictor 3.7*. After this, the approved molecules were imported into the software Osiris DataWarrior 5.0 [4] to estimate the risks of cytotoxicity based on four parameters: (mutagenicity, carcinogenicity, toxic effect on the reproductive system and skin irritability). Thus, the molecules that did not present any risk of cytotoxicity were considered.

Moreover, molecular docking was performed using the software Molegro Virtual Docker 6.0 [5] to calculate the energies of total ligand-receptor interactions with all chosen proteins (PDB ID 5ZNJ [6] and PDB ID 6N1X [7]), rank the thiophenics tested with some bactericidal drugs used as controls in this study and analyze the types of interactions involved between the molecules and the active sites of each protein used.

Results and Discussion

As mentioned previously, the molecules were tested in a biological activity prediction model and the data obtained can be seen in Table 1 below:

Table 1. Prediction results of biological activity against S. aureus.

ID	Domain	ATV	%ATV	ID	Domain	ATV	%ATV
AUR01	reliable	A	78.79	AUR16	reliable	A	55.25
AUR02	reliable	A	79.46	AUR17	reliable	A	55.25
AUR03	reliable	A	78.71	AUR18	reliable	A	53.50
AUR04	reliable	A	75.54	AUR19	reliable	I	45.00
AUR05	reliable	A	75.43	AUR20	reliable	A	78.76
AUR06	reliable	A	67.47	AUR21	reliable	A	76.84
AUR07	reliable	A	73.93	AUR22	reliable	A	73.08
AUR08	reliable	A	64.33	AUR23	reliable	A	77.42
AUR09	reliable	A	58.00	AUR24	reliable	A	70.83
AUR10	reliable	A	54.13	AUR25	reliable	A	76.75
AUR11	reliable	A	51.00	AUR26	reliable	A	72.78
AUR12	reliable	A	56.00	AUR27	reliable	A	68.13
AUR13	reliable	A	51.75	AUR28	reliable	A	57.63
AUR14	reliable	A	52.50	AUR29	reliable	A	62.13
AUR15	reliable	A	51.50	AUR30	reliable	A	79.46

ATV = Predicted biological activity
%ATV = ATV probability percentage

According to the model results, only the compound AUR19 showed no activity. The other molecules showed activity and were also approved by the applicability domain, making the predicted data reliable.

Another form of reliability of the model is its statistical data, in this research the developed model presented good results, since it demonstrated good precision (0.90). Sensitivity is the ability to evaluate a data as positive where it is really positive, as can be seen in the Table 2, the sensitivity in the internal

validation was 0.79 while in the test it was 0.80. Therefore, it can be said that the model is capable of defining true positive molecules with an acceptable degree of accuracy for a prediction model, with ROC curves above 0.80 for the internal validation and test groups (Figure 1).

Table 2. Statistical data of the generated model.

ID	Cross	Test
Precision	0.90	0.90
Sensitivity	0.79	0.80
Specifity	0.91	0.91
Accuracy	0.85	0.85
MCC	0.71	0.71

Cross = Internal Cross Validation Group
Test = Test group
MCC = Matthews Correlation Coefficient

One statistic that is worth commenting on is specificity, which is the ability of the model to classify molecules into inactive compounds when they truly lack activity. In the generated model, the validation and the test group have 0.91. Besides that, both accuracy and MCC were greater than 0.70, indicating that the overall accuracy of the model is good, as well as the premeditated overall capacity of the model.

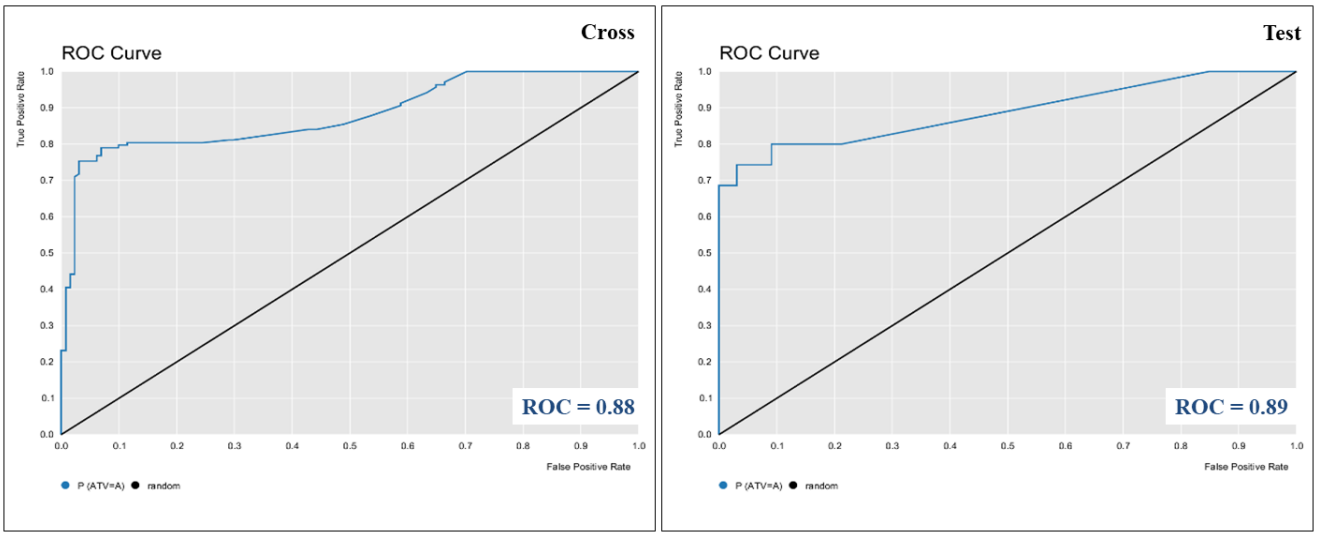


Figure 1. ROC curve of the model.

Following the discussions of the data generated in this research, the predictions of cytotoxic parameters of the compounds under study are shown in the Table 3. Only the compounds AUR07, AUR08 and AUR25 demonstrated risks in some of the four parameters, having total cytotoxicity. As the risk of mutagenicity was present in all three compounds, they do not have a promising therapeutic profile.

Table 3.Cytotoxicity risk prediction data.

ID	MUT	CAR	ESR	IRR	TOX	ID	MUT	CAR	ESR	IRR	TOX
AUR01	none	none	none	none	No	AUR16	none	none	none	none	No
AUR02	none	none	none	none	No	AUR17	none	none	none	none	No
AUR03	none	none	none	none	No	AUR18	none	none	none	none	No
AUR04	none	none	none	none	No	AUR19	none	none	none	none	No
AUR05	none	none	none	none	No	AUR20	none	none	none	none	No
AUR06	none	none	none	none	No	AUR21	none	none	none	none	No
AUR07	low	high	high	high	Yes	AUR22	none	none	none	none	No
AUR08	high	high	none	low	Yes	AUR23	none	none	none	none	No
AUR09	none	none	none	none	No	AUR24	none	none	none	none	No
AUR10	none	none	none	none	No	AUR25	high	none	none	none	YES
AUR11	none	none	none	none	No	AUR26	none	none	none	none	No
AUR12	none	none	none	none	No	AUR27	none	none	none	none	No
AUR13	none	none	none	none	No	AUR28	none	none	none	none	No
AUR14	none	none	none	none	No	AUR29	none	none	none	none	No
AUR15	none	none	none	none	No	AUR30	none	none	none	none	No

MUT = Multagenicity; CAR = Carcinogenicity; ESR = Toxic effect on reproductive system; IRR = Skin irritation and TOX = Total cytotoxicity.

Regarding molecular docking, this was done in thiophene derived compounds and 16 bactericides to serve as controls in this study, The proteins used were the PDB ID 5ZNJ corresponding to Proline-tRNA ligase (ProRS) and PDB ID 6N1X corresponding to glycosyltransferase (BshA) whose interaction energy data with the molecules under study are described in Table 4:

Tabela 4. Values of ligand-receptor interaction energies.

ID	PDB ID 5ZNJ [kcal.mol^{-1}]	PDB ID 6N1X [kcal.mol^{-1}]	ID	PDB ID 5ZNJ [kcal.mol^{-1}]	PDB ID 6N1X [kcal.mol^{-1}]
C01	-72.14	-81.68	AUR07	-81.26	-98.07
C02	-82.21	-80.66	AUR08	-76.16	-112.56
C03	-76.05	-88.67	AUR09	-99.56	-106.06
C04	-61.40	-88.60	AUR10	-97.94	-109.57
C05	-95.40	-82.95	AUR11	-106.28	-78.98
C06	-85.86	-98.62	AUR12	-77.02	-104.18
C07	278.29	819.19	AUR13	-96.62	-76.69
C08	-87.73	-101.43	AUR14	-79.09	-78.86
C09	-86.06	-97.23	AUR15	-103.68	-49.49
C10	-100.80	-99.49	AUR16	-111.63	-105.60
C11	-107.92	-97.88	AUR17	-67.11	-108.61
C12	-85.23	-169.57	AUR18	-71.87	-1.02
C13	-101.33	-60.78	AUR19	-84.99	-79.67
C14	-115.00	217.94	AUR20	-85.67	-103.75
C15	-34.02	-143.69	AUR21	-80.86	-88.62
C16	-22.89	331.33	AUR22	-76.84	-83.86
AUR01	-70.10	-80.13	AUR23	-54.56	-75.67
AUR02	-52.29	-68.77	AUR24	-52.46	-68.00
AUR03	-70.60	-85.40	AUR25	-51.87	-24.78
AUR04	-67.03	-82.94	AUR26	-39.90	-49.41

AUR05	-75.10	-90.49	AUR27	-40.99	-41.44
AUR06	-76.74	-87.84	AUR28	-60.24	-77.80
AUR07	-81.26	-98.07	AUR29	-63.26	-66.98
AUR08	-76.16	-112.56	AUR30	-59.66	-66.08
Inhibitor	-93.75	-78.42			
RMSD	1.44	0.88			

The data from the previous table are graphed in Figure 2, that demonstrates the ranking between the molecules tested, the controls used and the inhibitors complexed with each chosen protein. Thus, it it is possible to see that Control C07 showed no interaction in any of the proteins tested, nor did controls C14 and C16 for the BshA.

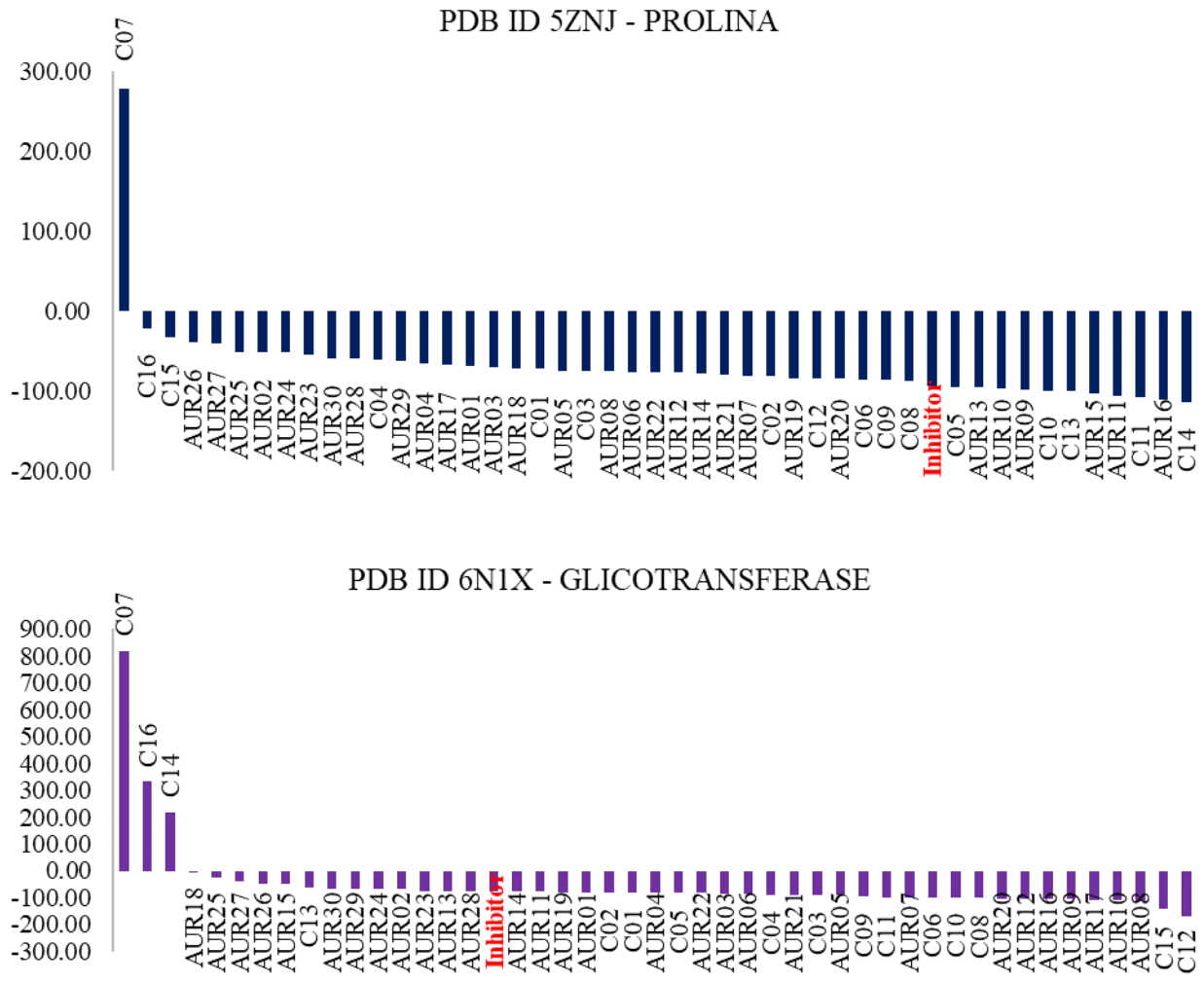


Figure 2. Rank chart of thiophenic energies, controls and inhibitors complexed together with each protein (red color).

Furthermore, it can be seen from the previous figure of ProRS that compounds AUR02, AUR23, AUR24, AUR25, AUR26, AUR27, AUR27, AUR28 and AUR30 showed worse ligand-receptor energy results than all controls, including controls C15 and C16 (-34.02 kcal.mol^{-1} and -22.89 kcal.mol^{-1}, respectively). This indicates that ProRS is not a possible target for these molecules, however, it is worth remembering that the compound AUR25 showed high mutagenicity according to the analyzes exposed

earlier in this research. For ProRS, AUR16 (-111.63 kcal.mol^{-1}) showed better energy results than 15 of the 16 controls used, including the complexed inhibitor (-93.75 kcal.mol^{-1}).

For BshA, compounds AUR08, AUR09, AUR10, AUR12, AUR16, AUR17 and AUR20 showed better results in molecular docking (between thiophenics). In fact, their results were better than 14 of the 16 controls used and the protein complexed ligand in the PDB. Corroborating with the prediction model results, all molecules classified as active showed interaction with the two proteins used, thus indicating the possible biological activity against *S. aureus*. Importantly, among the molecules tested for BshA, the compound AUR08 had the lowest energy, having the best interaction (-112.56 kcal.mol^{-1}), however, this compound presented cytotoxicity risks. Therefore, the best profile compound of this protein is AUR10 (-109.57 kcal.mol^{-1}) better than the complexed inhibitor (-78.42 kcal.mol^{-1}).

Conclusions

Of the thirty molecules tested, only 4 did not show a good pharmacochemical profile (AUR07, AUR08, AUR19 and AUR25) against the bacteria studied, and these compounds are possible bactericidal bioactive against *S. aureus*. Thus, it is expected to contribute to synthesis research groups by encouraging them to obtain these substances and further validation of the activity through *in vitro*/*in vivo* biological testing.

In general, the molecules tested are promising candidates for antibacterial drugs because they showed good energy results in molecular docking. Moreover, in both proteins studied there were compounds with better results than many commercial drugs used as controls, including the inhibitor complexed with each selected protein.

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