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Computational analysis of Macrolides as SARS-CoV-2 Main Protease Inhibitors: A Pattern Recognition Study Based on Molecular Topology and validated by Molecular Docking

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Table S1. Leave-some-out validation test for DF_1 by applying the criteria of leaving the 25% of the data set out as test set.

L-S-O nº	**λ**	**F**	**p <**	**HIV protease inhibition model (test set)**	**Percentage of Correct Class. (%)**
1	0.433	10.450	0.0012	Cinoxolone- Cyclosporin- Darunavir- Fuladectin A4- Lopinavir - Romifidine-	100
2	0.398	11.350	0.0010	Amprenavir- Ciluprevir- Epirubicin- Fosamprenavir- Ioglicic acid- Tenofovir disoproxil	67
3	0.393	11.575	0.0009	Apramycin-Atazanavir- Indinavir- Meclocycline Sulfosalicylate – Pranlukast - Tenofovir disoproxil	83
4	0.447	9.292	0.0024	Cromoglicate- Darunavir- Epirubicin- Indisulam- Picrotoxinin- Ritonavir	83
5	0.392	11.642	0.0009	Ciluprevir-Cinoxolone- Cyclosporin-Fosamprenavir – Fuladectin A4-Tipranavir	83
6	0.414	10.609	0.0013	Candoxatril- Cyclosporin- Darunavir- Indinavir-Indisulam- Metergotamine	100
7	0.402	11.148	0.0011	Apramycin-Atazanavir- Ioglicic acid- Pranlukast- Ritonavir- Tenofovir disoproxil	67
8	0.407	10.930	0.0012	Amprenavir- Ciluprevir- Cromoglicate - Feneritrol- Meclocycline - Tipranavir	100
9	0.424	10.177	0.0016	Apramycin- Amprenavir- Candoxatril - Fuladectin A4- Lopinavir- Metergotamine	83
10	0.383	12.092	0.0007	Cyclosporin-Darunavir- Feneritrol-Fosamprenavir- Picrotoxinin- Tenofovir disoproxil	83
Average	**0,409**	**10,927**	**0,0012**		**85**
DF_1	**0.449**	**14.751**	**0.0001**		

Table S2. Leave-some-out validation test for DF_2 by applying the criteria of leaving the 25% of the data set out as test set.

L-S-O nº	λ	F	p <	Hepatitis C protease inhibition model (test set)	Percentage of Correct Class. (%)
1	0.168	34.685	0.0006	Asunaprevir- Meclocycline-Simeprevir	100
2	0.222	24.521	0.0016	Epirubicin- Fuladectin A4-Paritaprevir	100
3	0.235	22.768	0.0020	Boceprevir- Paritaprevir.-Telaprevir	100
4	0.228	23.639	0.0018	Asunaprevir- Boceprevir - Metergotamine	100
5	0.264	19.542	0.0031	Boceprevir- Candoxatril-Telaprevir	100
6	0.256	20.377	0.0028	Boceprevir--Epirubicin- Fuladectin A4	100
7	0.163	36.009	0.0005	Ciluprevir- Meclocycline-Metergotamine	100
8	0.209	26.462	0.0013	Grazoprevir- Meclocycline-Paritaprevir	100
9	0.173	33.435	0.0007	Asunaprevir- Candoxatril-Simeprevir	100
10	0.220	24.874	0.0016	Fuladectin A4-Meclocycline- Paritaprevir	100
Average	**0,214**	**26,631**	**0,0016**		**100**
DF_2	**0.204**	**39.134**	**0.00009**		

Table S3. Leave-some-out validation test for DF_3 by applying the criteria of leaving the 25% of the data set out as test set.

L-S-O nº	λ	F	p <	SARS-CoV-2 protease inhibition model (test set)	Percentage of Correct Class. (%)
1	0.225	24.057	0.00001	Elbasvir-Feneritrol-Lopinavir-Metergotamine	100
2	0.187	30.371	0.00001	Enocitabine- Oftasceine - PB-88143175 - Teniposide	100
3	0.265	19.368	0.0001	Cromoglicate-Feneritrol-Remdesivir-Valrubicin	100
4	0.199	28.265	0.00001	Cinoxolone-Lopinavir-Meclocycline Sulfosalicylate-Remdesivir	100
5	0.223	24.358	0.00001	Cronidipin- Cyclosporin-Eravaccycline - PB-23727975	100
6	0.212	26.037	0.00001	Cronidipin-Enocitabine-Oftasceine-PB-88143175	100
7	0.265	19.427	0.0001	Carfilzomib-Cromoglicate-Elbasvir-Teniposide	100
8	0.146	40.878	0.00001	Enocitabine-Eravaccycline-Metergotamine-PB-88143175	100
9	0.277	18.278	0.0001	Elbasvir–Feneritrol-Oftasceine-Meclocycline Sulfosalicylate	100
10	0.246	21.485	0.0001	Cronidipin-PB-88143175-Teniposide-Valrubicin	100
Average	**0,439**	**10,531**	**0,0008**		**100**
DF_3	**0.236**	**29.108**	**0.00001**		

Table S4. Descriptor values for macrolides as potential protease inhibitors predicted by DF_{1-3}.

Compound	HIV protease inhibition model (DF_1)		Hepatitis C protease inhibition model (DF_2)	SARS-CoV-2 protease inhibition model (DF_3)	
	IC2	**SpMaxA_D/Dt**	**MATS6e**	**MATS6i**	**GATS7v**
Azithromycin	4,727	0,49	-0,027	0,123	1,132
Cethromycin	5,217	0,464	-0,037	0,124	1,095
Clarithromycin	4,661	0,521	-0,024	0,115	1,122
Diproleandomycin	4,812	0,561	-0,035	0,08	1,107
Erythromycin	4,661	0,521	-0,033	0,151	1,172
Fluritromycin	4,708	0,521	-0,025	0,103	1,175
Lexitromycin	4,724	0,528	-0,006	0,12	1,119
Mirosamicin	5,168	0,532	-0,056	0,08	1,013
Neutramycin	4,99	0,524	-0,022	0,036	1,023

3. Molecular docking on HIV and HCV main protease for macrolides understudy

A control simulation study on HIV (PDB: 4DJO) and HCV (PDB: 4A92) proteases has been made for macrolides understudy. According to literature, the conserved catalytic triad residues active sites for HIV protease are respectively: ASP25, THR26, and GLY27 (for first monomer of HIV protease) and ASP25′, THR26′ and GLY27′ (for second monomer of HIV protease) [1-2]. Whether catalytic pocket for HCV protease is formed by HIS528, MET485, GLN526, VAL524 and ALA625 [3-4].

As reported in the table S6, the results of the docking simulation using SwissDock on 4DJO show weak interactions with the catalytic site for the macrolides selected by molecular topology, with the exception of Mirosamycin. Catalytic residues of the active pocket are highlighted in bold font.

Table S5. Results for macrolides' molecular docking analysis on HIV main protease.

Molecular docking analysis on HIV main protease. [a]				
COMPOUND	**Estimated ΔG (kcal/mol)**	**HB**[b]	**Residue**	**HB distance (Å)**
Protease inhibitors				
Azithromycin	-5.7	0	n.a.	n.a.
Clarithromycin	-7.7	1	GLY48	1.722
Erithromycin	-6.4	1	LYS55	2.294
Lexithromycin	-6.2	0	n.a.	n.a.
Cetromycin	-6.6	0	n.a.	n.a.
Diproleandomycin	-8.5	1	ASP29	1.967
Fluritromycin	-7.9	1	GLY48	2.53
Mirosamycin	-9.5	3	**ASP25**	2.409
				2.008
			ASP29	1.885
Neutramycin	-10.1	3	ARG8	2.235
			GLY48	2.019
			ASP29	2.185
[a] The protein structure 4DJO retrieved from the Protein Databank (PDB) was used.				
[b] HB, hydrogen bonds.				
[c] Co-cristallized ligand				
[d] Reference compound.				

On the other hand, even if good results in terms of estimated ΔG (kcal/mol) are found when performing the docking simulation for HCV (all selected molecules show values of free energy at least below -8.5 kcal/mol - table S6), no Hb bonds with specific residues of the catalytic active site (in bold) are observed.

Table S6. Results for macrolides' molecular docking analysis on HCV main protease.

Molecular docking analysis on HCV main protease. [a]				
COMPOUND	**Estimated ΔG (kcal/mol)**	**HB[b]**	**Residue**	**HB distance (Å)**
Protease inhibitors				
Azithromycin	-8.7	2	GLN446	2.214
			ASN142	2.243
Clarithromycin	-8.9	1	GLN446	2.480
Erithromycin	-9.4	3	GLY449	2.355
			GLN446	2.113
				2.687
Lexithromycin	-9.4	1	ASN142	2.487
Cetromycin	-9.8	1	HSD95	1.827
Diproleandomycin	-10.6	3	ARG394	2.467
			GLN446	2.174
			ASP559	2.186
Fluritromycin	-9.8	1	ASN142	2.547
Mirosamycin	-10.1	3	ALA97	2.203
			ASP559	2.639
			GLN446	2.742
Neutramycin	-9.3	0	n.a.	n.a.
[a] The protein structure 4A92 retrieved from the Protein Databank (PDB) was used.				
[b] HB, hydrogen bonds.				
[c] Co-cristallized ligand				
[d] Reference compound.				

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