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Laurent Soulère, Thibaut Barbier, Yves Queneau. Docking-based virtual screening studies aiming at the covalent inhibition of SARS-CoV-2 MPro by targeting the cysteine 145. *Computational Biology and Chemistry*, 2021, 92, pp.107463. 10.1016/j.compbiolchem.2021.107463 . hal-03243702

HAL Id: hal-03243702

<https://hal.science/hal-03243702>

Submitted on 31 May 2021

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1 **Docking-based virtual screening studies aiming at the covalent inhibition of**
2 **SARS-CoV-2 M^{Pro} by targeting the cysteine 145**

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8
9 KEYWORDS. SARS-CoV-2; Covalent inhibitors; M^{Pro}; flexible docking; virtual screening.

10

11

12 **ABSTRACT**

13 The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is responsible for the
14 COVID-19 which has infected millions of people worldwide. The main protease of SARS-CoV-
15 2 (M^{Pro}) has been recognized as a key target for the development of antiviral compounds. Taking
16 advantage of the X-ray crystal complex with reversible covalent inhibitors interacting with the
17 catalytic cysteine 145 (Cys145), we explored flexible docking studies to select alternative
18 compounds able to target this residue as covalent inhibitors. First, docking studies of three
19 known electrophilic compounds led to results consistent with co-crystallized data validating the
20 method for SARS-CoV-2 M^{Pro} covalent inhibition. Then, libraries of soft electrophiles (overall
21 41 757 compounds) were submitted to docking-based virtual screening resulting in the
22 identification of 17 molecules having their electrophilic group close to the Cys145 residue. We
23 also investigated flexible docking studies of a focused approved covalent drugs library including
24 32 compounds with various electrophilic functional groups. Among them, the calculations
25 resulted in the identification of four compounds, namely dimethylfumarate, fosfomycin, ibrutinib
26 and saxagliptin, able first, to bind to the active site of the protein and second, to form a covalent
27 bond with the catalytic cysteine.

28

29

30 INTRODUCTION

31 COVID-19 is an infectious disease caused by the severe acute respiratory syndrome coronavirus
32 2 (SARS-CoV-2).(Lai et al., 2020) In November 2020, this disease has infected more than
33 55 300 000 people worldwide, including more than 1 300 000 deaths (<https://covid19.who.int/>).
34 Consequently, there is an urgent need to identify new anti-viral drugs targeting this virus. Several
35 strategies for identifying coronavirus anti-viral drugs have been described in the literature and
36 they have been recently reviewed by Thanigaimalai Pillaiyar and co-workers.(Pillaiyar et al.,
37 2020) Among them, an important approach consist in inhibiting the SARS-CoV-2 main protease
38 (M^{pro}) by peptide mimics or other types of compounds.(Lu et al., 2006; Pillaiyar et al., 2016)
39 Several studies have been devoted to computational determination of potential inhibitors of the
40 SARS-CoV-2 main protease such as computational drug repurposing studies,(Arun et al., 2020;
41 Wang, 2020) structure-based virtual screening studies(Gahlawat et al., 2020; Ton et al., 2020)
42 and docking studies of natural compounds.(Gentile et al., 2020; Ngo et al., 2020) A strategy of
43 achieving irreversible inhibition of this protease has also been addressed by the design of
44 compounds to create a covalent bond with the cysteine 145 residue (Cys145) of the catalytic
45 dyad.(Pillaiyar, et al., 2020) While classical docking studies are widely reported in the
46 literature,(Kitchen et al., 2004) docking studies for covalent protein inhibition are less
47 common,(Kumalo et al., 2015; Sotriffer, 2018) in particular with SARS-CoV-2 main
48 protease.(Liu et al., 2020; Paul et al., 2020) Despite covalent inhibition approaches are less
49 studied because the requirement of a nucleophilic residue is a structural limitation and they can
50 be considered as harmful, the resurgence of covalent drugs encourage to also consider covalent
51 inhibition.(Dalton et al., 2020; Ghosh et al., 2019; Singh et al., 2011) Irreversible specific protein
52 inhibitors are now reported, such as in the case of the Ras protein possessing a G12C mutation, a

61 **Figure 1.** Structure, representation of the alpha-ketoamide inhibitor 1 within the M^{Pro} active site
62 with surface and hydrogen bonds network.(L. Zhang, et al., 2020)

63

64 **MATERIALS AND METHODS**

65 *Proteins used in the present study*

66 The main protein of SARS-CoV-2 M^{Pro} was obtained from the protein data bank (PDB code
67 6Y2F, 5RHF, 5REN, 5REK).

68 *Docking studies of known covalent inhibitors 2-4 with crystallographic data*

69 Compounds **2-4** were generated as 3D mol file using Arguslab(Thompson, 2004) and they were
70 then docked within the M^{Pro} active site (PDB codes 5RHF, 5REN, 5REK respectively) using
71 Arguslab software with the Argusdock engine with default parameters. The obtained binding
72 modes were compared to crystallographic data to validate the method. The distance between the
73 sulfur and the methylene group of the chloroacetamide group was monitored to establish the
74 structural bases of the M^{Pro} covalent inhibition (distance < 4 Å).

75 *Docking-based virtual screening of potential covalent inhibitors*

76 Compounds libraries were obtained from PubChem using structure search of acrylamide,
77 halogenoacetamide, cyanoacrylamide and vinylsulfonamide. Asinex and Enamine soft
78 electrophilic compounds libraries were obtained from the corresponding website and hydrogen
79 atoms were added using OpenBabel 2.4.1..(O'Boyle et al., 2011) Each compound from the
80 different libraries (SDF file) were then docked within the M^{Pro} active site centered on the β -keto

81 amide **1** using Arguslab software with the Argusdock engine with default parameters. Based on
82 their docking score, binding modes were analyzed for the 20 best compounds of each library.
83 The distance between the sulfur and the electrophilic center was monitored and compounds were
84 selected when a distance $< 4 \text{ \AA}$ was measured. Binding modes were examined with PyMOL and
85 hydrogen bonds networks were generated using LigPlot +.(Laskowski et al., 2011) For all
86 selected compounds, further flexible docking experiments with a genetic algorithm engine
87 implemented in Arguslab were achieved to corroborate the docking results (see supplementary
88 information).

89 *Docking studies of the approved covalent drugs library*

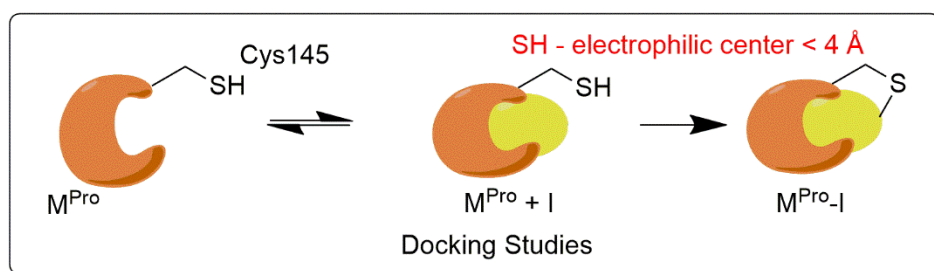
90 The library was constructed based on the list of covalent drugs established by Vasudevan and co-
91 workers,(Vasudevan et al., 2019) from which β -lactam derivatives, drugs withdrawn from
92 market and mechanism based covalent drugs were excluded. For five of the 29 remaining
93 compounds, their metabolites which are the active covalent binders(Shin et al., 2013) were used
94 in the library. Overall, 32 compounds were used for the docking studies. When available, the
95 SDF file for each compound was obtained from PubChem and was converted to PDB file using
96 Accelrys Visualizer 2.0. For omeprazole, lansoprazole, pantoprazole and rabeprazole active
97 metabolites, they were drawn using Vega ZZ(Pedretti et al., 2002, 2004) and were saved as PDB
98 files. The 32 compounds were then docked within the M^{Pro} active site centered on the β -keto
99 amide **1** using Arguslab software(Thompson, 2004) with the Argusdock engine with default
100 parameters. The distance between the sulfur and the electrophilic center was monitored and
101 compounds were selected when a distance $< 4 \text{ \AA}$ was measured. Binding modes were examined
102 with PyMOL and hydrogen bonds networks were generated using LigPlot +.(Laskowski and
103 Swindells, 2011) Further flexible docking experiments with a genetic algorithm engine

104 implemented in Arguslab were achieved to corroborate the docking results (see supplementary
105 information).

106

107 RESULTS AND DISCUSSION

108 When applicable, the search for covalent inhibitors by docking can be carried out according to
109 the scheme depicted in Figure 2 by targeting a nucleophilic residue such as a cysteine residue. It
110 is then necessary that the electrophilic center is placed at the vicinity of the thiol function of this
111 residue. This approach can be investigated with the M^{Pro} protein that contains a catalytic cysteine
112 residue (Cys145). In order to explore the potentialities to covalently inhibit M^{Pro} , a flexible
113 docking of compounds was performed to establish the structural bases for binding recognition
114 with high affinity to improve the selectivity to the molecular target to form the complex $M^{\text{Pro}} + I$.
115 In a second time, examination of the potentialities of the covalent inhibition of this protein due to
116 the formation of a covalent adduct $M^{\text{Pro}}-I$ can be examined. For this, the location of the
117 electrophilic moiety of compounds, in particular the distance between the electrophilic center
118 and the thiol of Cys145 has to be adequate (Y. Zhang et al., 2016) (Figure 2). As a consequence,
119 we used standard docking experiments targeting Cys145 with a cutoff of 4 Å as a theoretical
120 study.(Choi et al., 2016; Lin et al., 2011)

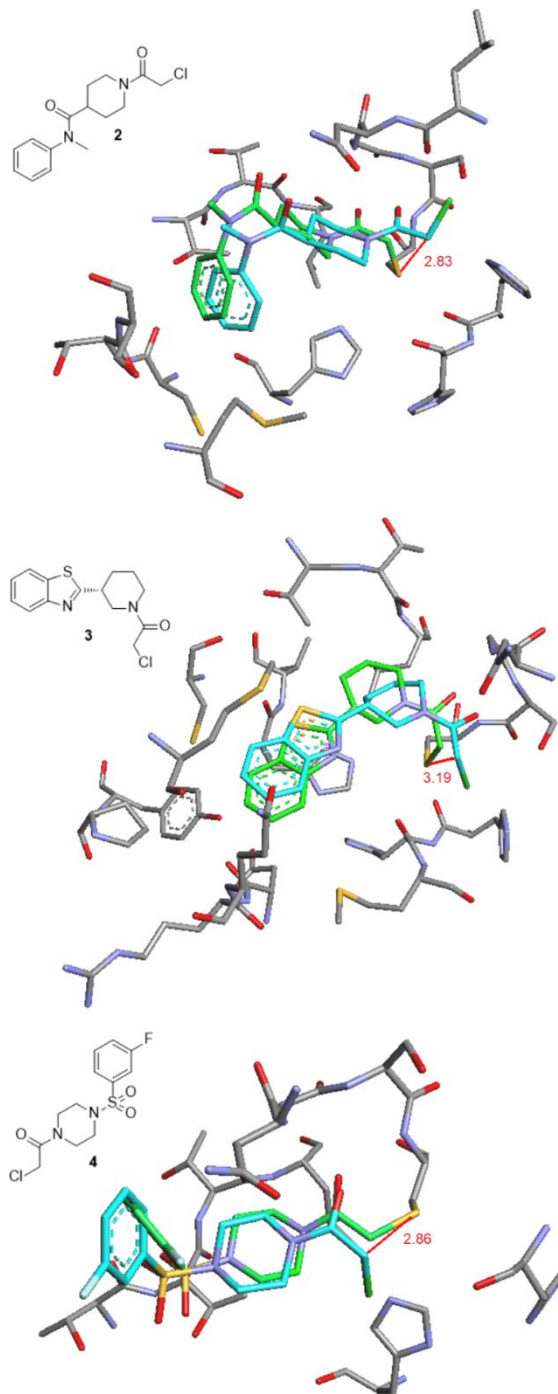


122 **Figure 2.** Docking studies for covalent inhibition of SARS-CoV-2 M^{Pro} by targeting the Cys145
123 residue.

124

125 *Docking studies of known covalent inhibitors of SARS-CoV-2 M^{Pro}*

126 In order to explore the potentialities to covalently inhibit SARS-CoV-2 M^{Pro}, we first studied
127 docking of the covalent inhibitors **2-4** co-crystallized within the protease to establish the
128 structural bases for the covalent inhibition of this protein.(Douangamath et al., 2020) In
129 particular, the location of the electrophilic moiety of compounds i.e. the distance between the
130 electrophilic center and the thiol of Cys145 was investigated. Docking experiments were thus
131 conducted on these three compounds; the results are depicted in Figure 3. These experiments
132 show consistent results with the crystallographic data (PDB codes 5RHF, 5REN, 5REK
133 respectively) and show that the electrophilic center of compounds i.e. the methylene group of the
134 chloroacetamide functional group is located at the vicinity of Cys145 with distance values
135 between the carbon and the thiol atoms ranging from 2.83 Å to 3.19 Å.



136

137 Figure 3: Docking results (colored in cyan) obtained from docking experiments conducted with
 138 compounds 2-4 within the active site of M^{pro} (co-crystallized compounds are indicated in green
 139 for comparison). Distance (Å) between the thiol function of Cys145 and the electrophilic center
 140 is shown in red color.

141
142 Based on these docking experiments of compounds **2-4**, we defined the structural basis for the
143 covalent inhibition of M^{pro} as 1) binding the protein with a good affinity and 2) having an
144 adequate orientation of the electrophilic moiety towards Cys145 with a distance inferior to 4 Å
145 with the sulfur (Figure 2).

146
147 *Docking-based virtual screening studies for covalent inhibition of M^{Pro}*

148 We then investigated docking-based virtual screening studies for covalent inhibition of M^{Pro}
149 using libraries of electrophilic inhibitors (Table 1). A total of 41 757 compounds in diverse
150 libraries constructed from PubChem or available from Enamine or Asinex were employed for
151 docking simulations. Docking poses for compounds ranked in the top 20 in each library were
152 then examined to evaluate the distance between the electrophilic center and the sulfur atom.

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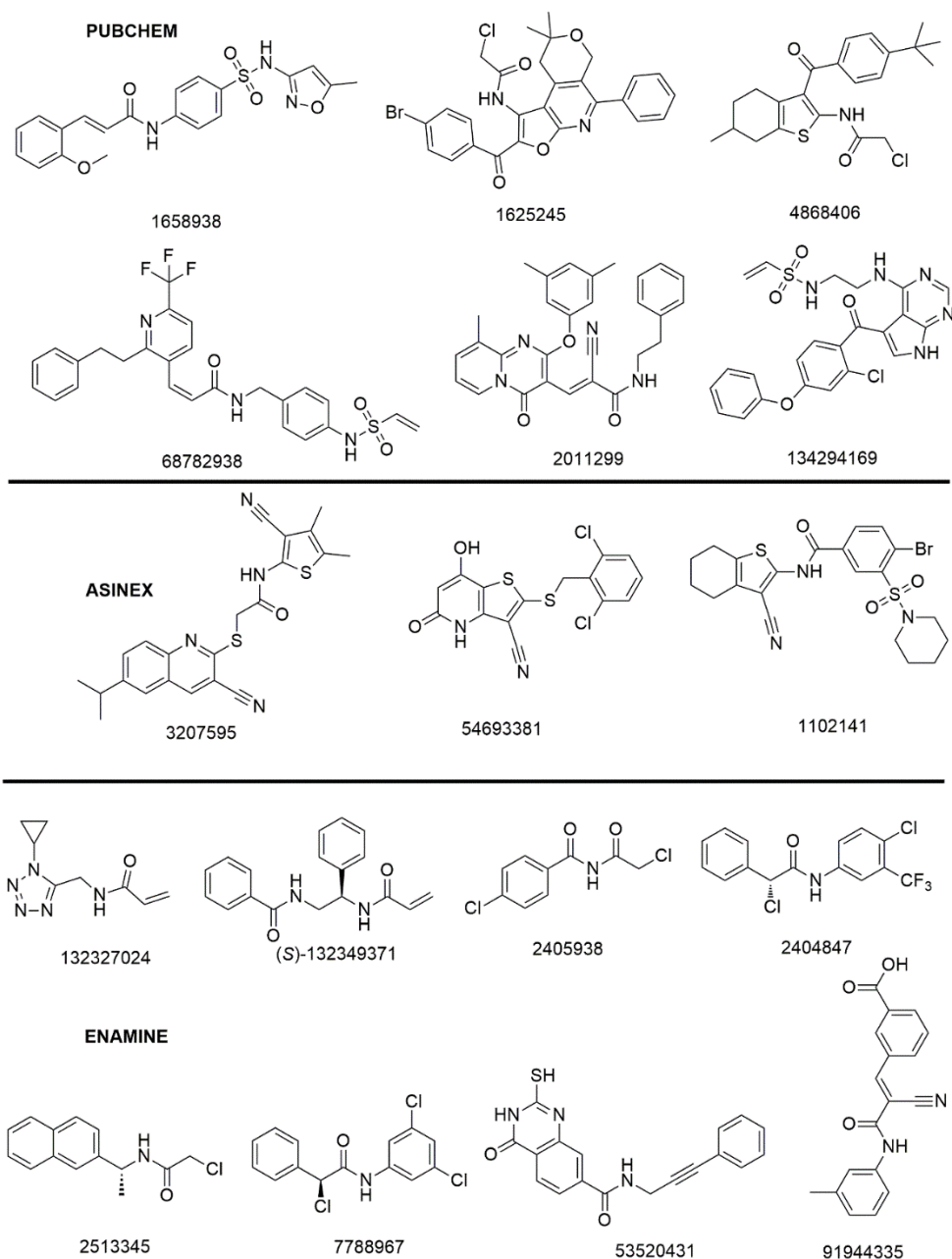
Table 1. Potential covalent inhibitors libraries used in this study.

Compounds libraries	Structures	Compounds number/potential inhibitors	Adducts
PubChem acrylamide		3905/1	Michael adduct
PubChem halogenoacetamide		4281/2	Nucleophilic substitution
PubChem cyanoacrylamide		3356/1	Reversible covalent adduct
PubChem vinylsulfonamide		1070/2	Michael adduct
Asinex Soft electrophile	Diverse	8098/3	Structure dependent
Enamine Covalent Screening Library	Diverse	15684/2	Structure dependent
Enamine Halogenoacetamide		2210/4	Nucleophilic substitution
Enamine Acrylamide		3153/2	Michael adduct

161

162 Structures of all potential covalent inhibitors of M^{Pro} are shown in Figure 4. All compounds were
 163 found to be aromatic ones with a high diversity of structures. Among the 17 compounds, three
 164 compounds are structurally related to acrylamides, two to vinylsulfonamides and six to

165 electrophilic chlorinated compounds. An alkyne derivative and three activated cyano-compounds
 166 could be also identified as well as two cyanoacrylamides. These latter compounds are
 167 particularly interesting leading to reversible covalent inhibition.(Serafimova et al., 2012)



168
 169 **Figure 4.** Structures of potential covalent inhibitors of M^{Pro} , with PubChem CID number.

170 Binding mode of each compound was carefully examined in terms of hydrogen bonds with the
 171 protein M^{Pro}, and the distance between the thiol and the electrophilic center was also measured
 172 (Table 2). Hydrogen bonds numbers were ranging from one to six for the compound 134294169.
 173 The docking score was found to be variable depending of the compounds library and the
 174 structure of the compounds. These values were ranging from – 7.22 to – 10.41 kcal.mol⁻¹ for the
 175 compound 1102141.

176

177 **Table 2.** Potential covalent inhibitors of M^{Pro} : docking score, hydrogen bonds numbers and Sn
 178 indicates the binding pockets interacting with the ligands (L. Zhang, et al., 2020), sulfur –
 179 electrophilic group distances.

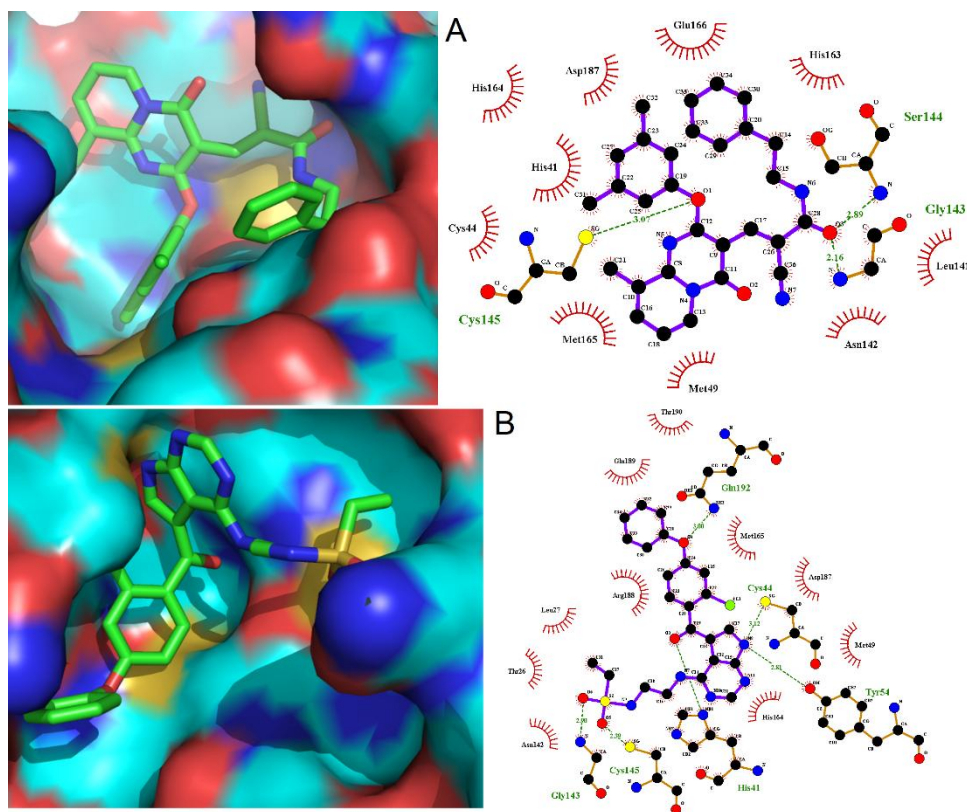
Compounds	Docking score (kcal.mol ⁻¹)	H-bonds numbers, Sn	Sulfur – electrophilic center distances (Å)
1658938	– 9.19	1, S1 S3 S4	3.62
1625245	– 9.83	3, S1 S3 S4	4.03
4868406	– 9.28	2, S1 S2 S4	3.40
68782938	– 9.21	5, S1 S2 S3 S4	3.48
2011299	– 10.32	3, S1 S2 S4	3.18
134294169	– 9.43	6, S1 S2 S3 S4	3.80
3207595	– 10.18	1, S1 S2 S4	3.46
54693381	– 9.99	3, S1 S2 S3	2.97
1102141	– 10.41	1, S1 S3 S4	3.82
132327024	– 7.22	3, S1	3.94

132349371	- 7.35	3, S1 S2 S3	3.76
2405938	- 8.19	3, S1 S2	3.27
2404847	- 7.94	1, S1 S2 S3	3.51
2513345	- 7.96	2, S1 S3	3.57
7788967	- 8.49	2, S1 S2 S3	3.89
53520431	- 8.81	3, S1 S3	2.27
91944335	- 8.39	4, S1 S2	3.99

180

181 As example, we chose to depict the binding modes of two compounds with high docking score
182 values. The binding modes of the compound 2011299, which exhibits a low distance between the
183 thiol and the electrophilic center and the one of the compound 134294169 with six hydrogen
184 bonds, are described in Figure 5. The two compounds fit well in the active site. The compound
185 2011299 interacts with the Gly143, Ser144 and Cys145 residues via hydrogen bonds and with a
186 distance of 3.18 Å between the sulfur and the electrophilic carbon atom of the cyanoacrylamide
187 functional group. This group is of particular interest by potentially promoting reversible covalent
188 inhibition. The compound 134294169 interacts tightly within the active site with the His 41,
189 Cys44, Tyr54, Gly143, Cys145 and Gln192 residues via six hydrogen bonds and with a distance
190 of 3.80 Å between the sulfur and the electrophilic carbon atom of the vinyl sulfonamide
191 functional group. We also mention the compound 1658938 with a valuable docking score and a
192 distance of 3.62 Å. Indeed, this compound shows a low cytotoxicity which has been
193 demonstrated in several bioassays (see PubChem bioassays 435019, 1825, 504648, 602141).

194



195

196 **Figure 5.** Binding modes of compounds 2011299 (A) and 134294169 (B) with the corresponding
 197 hydrogen bonds network.

198

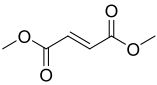
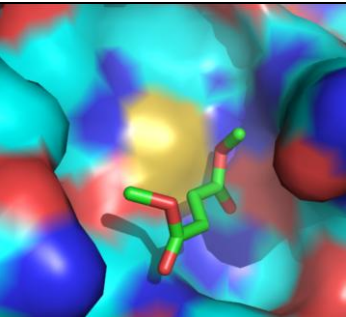
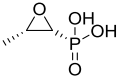
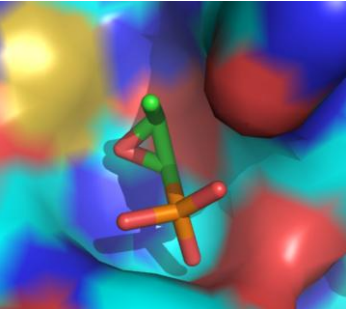
199 *Flexible docking studies of an approved covalent drugs library targeting the Cys145 residue*

200 Based on the approach described in Figure 2, we investigated a library of known approved
 201 covalent drugs. For this, we used a list of 52 FDA (Food and Drug administration) approved
 202 drugs described by Anil Vasudevan and colleagues in 2019.(Vasudevan, et al., 2019) Among
 203 these, we excluded some molecules for our study, such as drugs withdrawn from the market and
 204 drugs with a mechanism-based inhibition, resulting in a small library of 32 compounds. On this
 205 library, the workflow described in Figure 2 was applied by means of flexible docking

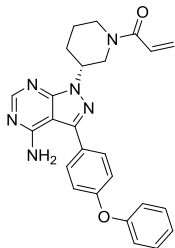
206 experiments and subsequent distance measurement between the Cys145 residue and the
 207 electrophilic center. Following this experiment, four compounds showed interesting results and
 208 they are presented in Table 3.

209

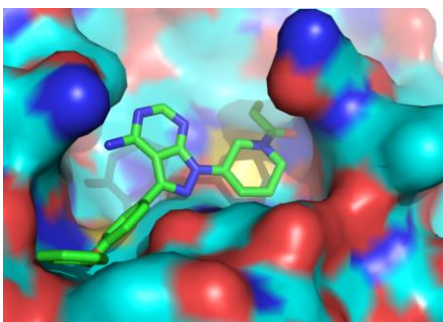
210 **Table 3.** Potential approved drugs as covalent inhibitors of M^{Pro}: binding modes, hydrogen
 211 bonds numbers and Sn indicates the binding pockets interacting with the ligands (L. Zhang, et
 212 al., 2020), thiol – electrophilic group distances.

Compounds (electrophilic moiety)	Binding modes	H- bonds, Sn	S – electrophilic center distances
Dimethylfumarate  (α,β -unsaturated ester)(Torkildsen et al., 2016)		4, S1	2.98 Å
Fosfomicin  (epoxide)(Michalopoulos et al., 2011)		3, S1	3.73 Å

Ibrutinib

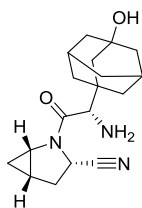


(acrylamide)(Burger et al., 2015)

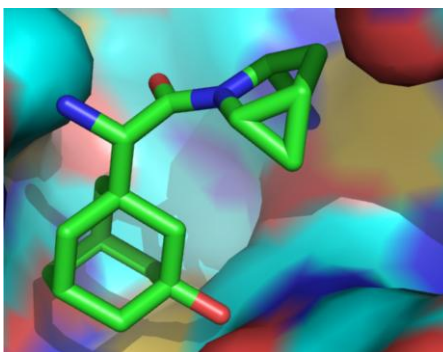


5,
S1 S3 3.39 Å
S4

Saxagliptin



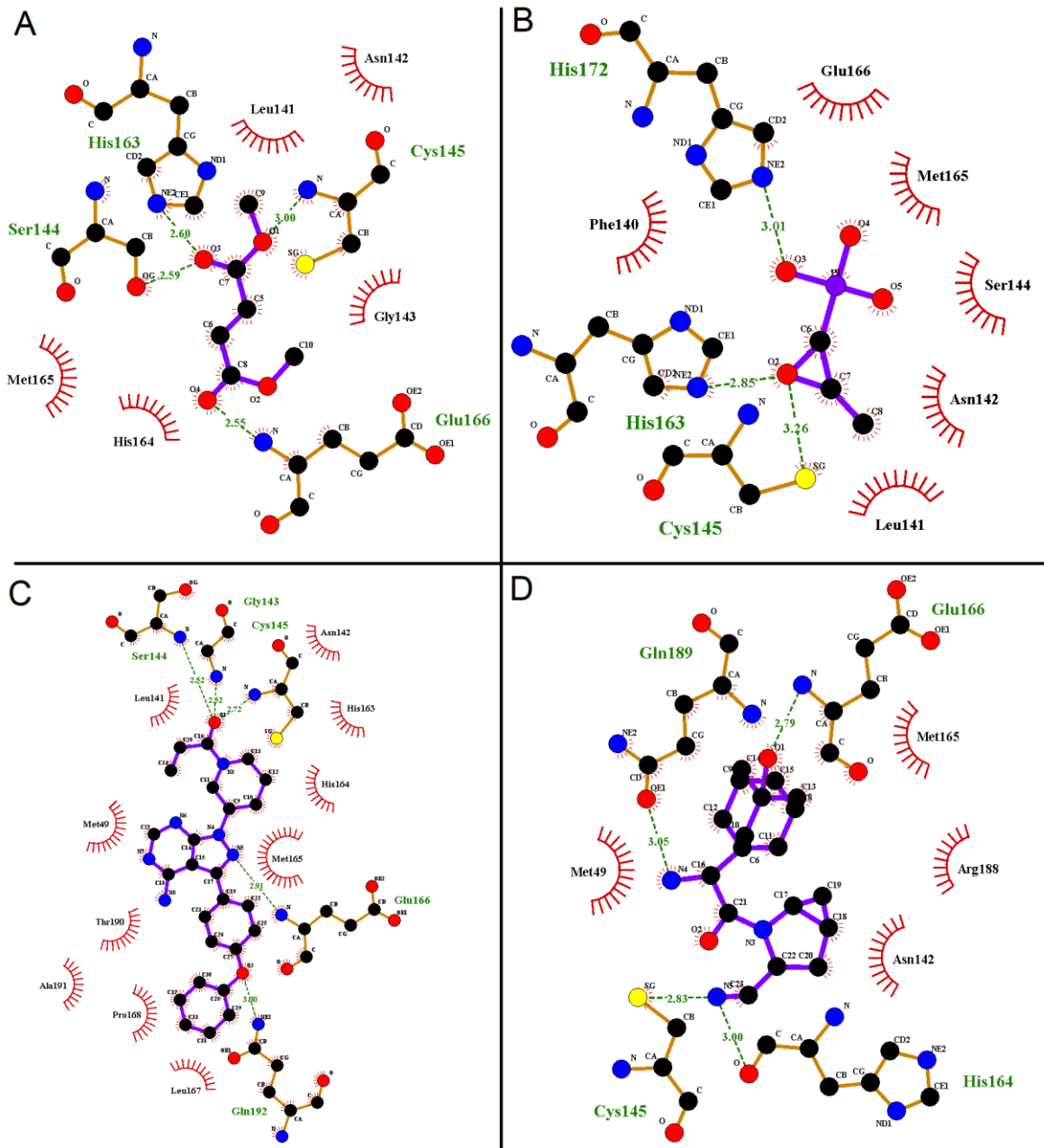
(nitrile)(Barnett, 2006)



4,
S1 S3 3.78 Å

213

214 Careful analysis of the proposed binding mode of these compounds with the corresponding
215 hydrogen bonds network revealed that they could be able to bind tightly within the M^{Pro} binding
216 site. The distance between the sulfur and the electrophilic center is ranging from 2.98 and 3.78 Å
217 showing their potentialities to act as covalent inhibitors. The 2D-representation is described in
218 Figure 6 for the four compounds, showing the hydrogen bonds networks.



219

220 **Figure 6.** 2D representation of the proposed binding modes of the four potential covalent
 221 inhibitors within the binding site of M^{Pro} : dimethylfumarate (A), fosfomycin (B), ibrutinib (C),
 222 saxagliptin (D).

223

224 These four compounds are approved drugs for a designated pathology (Table 4) and some
 225 literature data can be found about relationships between COVID-19 and these compounds. First,
 226 Vittorio Mantero and colleagues reported that the use of dimethylfumarate for patients suffering
 227 from multiple sclerosis seems to have a positive impact against COVID-19.(Mantero et al., 2020)
 228 For ibrutinib, two studies shows that this compound seems to have a protective role against
 229 COVID-19, although they hypothesize that it may be due to the anti-inflammatory effect of the
 230 Bruton’s tyrosine kinase pathway inhibition.(Thibaud et al., 2020; Treon et al., 2020) For
 231 saxagliptin, Alicja Krejner-Bienias and colleagues hypothesized that antidiabetic drugs, gliptins,
 232 could prevent the virus from binding to dipeptidyl peptidase IV (DPP IV)(Krejner-Bienias et al.,
 233 2020) Saxagliptin could then have a double effect on both M^{Pro} and DPP IV. For fosfomycin, no
 234 relationship between this compound and COVID-19 can be found in the literature to date.

235 **Table 4.** Biological target and therapeutic domain for the proposed compounds.

International nonproprietary name	Biological Target	Therapeutic Domain
Dimethylfumarate	NF-κB activation	Multiple sclerosis
Fosfomycin	UDP-N-acetylglucosamine-3-enolpyruvyltrans-ferase	Anti-infective
Ibrutinib	Bruton’s tyrosine kinase (BTK)	Cancer
Saxagliptin	Dipeptidyl peptidase IV (DPP IV)	Anti-diabetic drug

236

237

238

239 **CONCLUSION**

240 To summarize, we described docking studies directed toward the identification of potential
241 covalent inhibitors of SARS-CoV-2 M^{Pro} with significant structural diversity by targeting the
242 cysteine 145 residue. A docking-based virtual screening approach of 41 757 compounds
243 belonging to libraries of soft electrophilic small molecules, allowed us to unveil 17 more
244 potential covalent inhibitors. Then, using a library of approved covalent drugs, four compounds
245 namely dimethylfumarate, fosfomycin, ibrutinib and saxagliptin were identified for their
246 theoretical ability to first bind to the active site of the protein and second to form a covalent bond
247 with the catalytic cysteine. This study provides structural insights in the covalent inhibition of
248 M^{Pro}, which might be useful in the search for therapeutic approaches fighting COVID-19.

249

250 **Conflicts of Interest**

251 None to declare.

252

253 **ACKNOWLEDGMENT**

254 The authors are grateful to CNRS, MESRI for financial support and to the MESRI for a
255 scholarship to Thibaut Barbier.

256

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