The Discovery of Potential SARS-CoV-2 Natural Inhibitors among 4924 African Metabolites Targeting the Papain-like Protease: A Multi-Phase In Silico Approach

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Abstract: Four compounds, hippacine, 4,2′-dihydroxy-4′-methoxychalcone, 2′,5′-dihydroxy-4′-methoxychalcone, and wighteone, were selected from 4924 African natural metabolites as potential inhibitors against SARS-CoV-2 papain-like protease (PLpro, PDB ID: 3E9S). A multi-phased in silico approach was employed to select the most similar metabolites to the co-crystallized ligand (TTT) of the PLpro through molecular fingerprints and structural similarity studies. Followingly, to examine the binding of the selected metabolites with the PLpro (molecular docking. Further, to confirm this binding through molecular dynamics simulations. Finally, in silico ADMET and toxicity studies were carried out to prefer the most convenient compounds and their drug-likeness. The obtained results could be a weapon in the battle against COVID-19 via more in vitro and in vivo studies.

Keywords: African natural products; SARS-papain-like protease; molecular fingerprints; structural similarity; docking; ADMET; toxicity; molecular dynamics simulations

1. Introduction

On 11 November 2022, the WHO stated that the confirmed global infections of COVID-19 were 630,832,131, with 6,584,104 people dead [1]. Conforming to these gigantic numbers, massive work is demanded from scientists worldwide to find a cure.

The evolution of computational chemistry methods as a successful tool to conclude the physical and chemical properties of a molecule and the molecular reactions allowed deep identification of the molecular properties of compounds in addition to their interactions with different proteins [2]. Consequently, the in silico prediction of the activity of large libraries of compounds against a specific molecular target became available [3]. The computational (in silico) chemistry methods have been employed in drug discovery [4–6], molecular modeling [7], and design [8,9]. Additionally, it has been used to predict ADMET [10–12], toxicity [13–15] as well as DFT [16] properties.

The interest of humans in the use of natural products can be traced back hundreds of years and continues to the present [17,18].

The papain-like protease, PLpro, is a vital enzyme in the coronavirus. PLpro has an important role in the processing mechanism of viral polyproteins. This process leads to the
formation of an active replicase complex [19]. Besides, PLpro has an additional vital role in deactivating human immunity. PLpro acts on human enzymes via cleaving proteinaceous post-translational modifications [20].

Our teamwork employed the in silico approaches to explore the potentialities of natural products against COVID-19 several times before. The determination of the most convenient inhibitors between fifty-nine isoflavonoids against hACE2 and main viral protease has been reported [21]. Likely, the activities of a set of fifteen alkaloids against COVID-19 five enzymes have been published [22].

In the presented work, a set of 4924 African natural products (compounds isolated from African natural sources) has been selected. The experiment set was obtained from the African Natural Products Database (ANPDB), a collection of several natural product databases in different African regions. The selected data set covered the period of 1962–2019 and was derived from international and local African journals in addition to MSc and Ph.D. theses in African university libraries [23].

The selected compounds were screened using multistage computational methods to detect the most potent SARS-CoV-2 PLpro inhibitors. The applied methods included molecular structures similarity study against the co-crystallized ligand (TTT) of PLpro (PDB ID: 3E9S) [24], fingerprint study against the same ligand, molecular docking against PLpro, ADMET, toxicity and molecular dynamics (MD) simulation experiments.

2. Method

2.1. Molecular Similarity Detection

Discovery Studio 4.0 software, 2016, Vélizy-Villacoublay, France, was used to investigate the similarities between 4924 African natural metabolites and TTT, the co-crystallized ligand of PLpro (Supplementary Data provides comprehensive details).

2.2. Fingerprint Studies

Discovery Studio 4.0 software was used to investigate the similarities between 100 African natural metabolites and TTT, the co-crystallized ligand of PLpro (Supplementary Data provides comprehensive details).

2.3. Docking Studies

Docking studies were done for the most similar 40 metabolites against the PLpro protease (PDB ID: 3E9S) using Discovery studio software [25] to investigate the binding energies as well as binding modes (Supplementary Data provides comprehensive details).

2.4. ADMET Analysis

Discovery Studio 4.0 was used [26] to examine 5 different ADMET parameters for 17 metabolites of correct binding scores (Supplementary Data provides comprehensive details).

2.5. Toxicity Studies

Discovery Studio 4.0 software was used [27–29] to examine 7 different toxicity parameters for 7 metabolites of good ADMET profile (Supplementary Data provides comprehensive details).

2.6. Molecular Dynamics Simulation

The PLpro-wightcone system was prepared using the web-based CHARMM-GUI [30–32] interface utilizing the CHARMM36 force field [33] and NAMD 2.13 [34] package. The TIP3P explicit solvation model was used (Supporting Data provides comprehensive details).

3. Results and Discussion

3.1. Structure Fingerprints Study

The basic assumption of structure-activity relationship studies is that “Chemical compounds with similar structures may have similar activities” [35]. This assumption was very useful in discovering several bioactive ligands [36]. The high affinity of the co-crystallized
ligand to bind with the targeted protein was our main motive in this work. We utilized some ligand-based computational techniques such as structure fingerprints and similarity to select the natural compounds (through the examined library) that have high degrees of similarities and hence could bind with PLpro effectively. The fingerprint study is a molecular descriptor technique widely used to figure out the similarity or dissimilarity between the chemical structures of two molecules or more [37,38]. In fingerprint study, the software converts the basic chemical molecular descriptors into mathematical symbols. The resulting data is displayed as bit strings that identify the presence (1) or absence (0) of a specific 2D atomic or fragment descriptor in both test and reference compounds [39,40]. In this study, Discovery Studio software examined the molecular fingerprints of 4924 compounds against TTT. This study aims to extract the most similar natural compounds to the ligand. The employed descriptors are H-bond acceptor [41] and donor [42], charge [43], hybridization [44], positive [45] and negative ionizable atoms [46], halogens [47], aromatic [48], or none of the above besides the ALogP [49] category of fragments and atoms. The study was adjusted to choose the most structurally similar 200 compounds to TTT (Table 1).

Table 1. Fingerprint similarity between the tested African metabolites and TTT.

| Compound | Similarity | SA  | SB  | SC  | Compound | Similarity | SA  | SB  | SC  |
|----------|------------|-----|-----|-----|----------|------------|-----|-----|-----|
| TTT      | 1.000      | 454 | 0   | 0   | 3448     | 0.632653   | 279 | −13 | 175 |
| 2538     | 0.758      | 322 | −29 | 132 | 3647     | 0.632479   | 296 | 14  | 158 |
| 3518     | 0.747      | 324 | −20 | 130 | 292      | 0.632249   | 447 | 253 | 7   |
| 3323     | 0.743      | 324 | −18 | 130 | 1795     | 0.632054   | 280 | −11 | 174 |
| 2982     | 0.743      | 329 | −12 | 125 | 3414     | 0.631699   | 554 | 423 | −100|
| 2981     | 0.738      | 327 | −11 | 127 | 2259     | 0.631188   | 255 | −50 | 199 |
| 182      | 0.732      | 314 | −25 | 140 | 3040     | 0.63035    | 324 | 60  | 130 |
| 2677     | 0.720      | 317 | −1  | 137 | 1157     | 0.630081   | 310 | 38  | 144 |
| 2558     | 0.712      | 442 | 167 | 12  | 1141     | 0.629797   | 279 | −11 | 175 |
| 2554     | 0.710      | 316 | −9  | 138 | 2180     | 0.62963    | 255 | −49 | 199 |
| 1875     | 0.710      | 320 | −3  | 134 | 203      | 0.628889   | 283 | −4  | 171 |
| 197      | 0.708      | 334 | 18  | 120 | 3413     | 0.628831   | 554 | 427 | −100|
| 1168     | 0.703      | 298 | −30 | 156 | 2108     | 0.628062   | 282 | −5  | 172 |
| 2556     | 0.703      | 298 | −30 | 156 | 1332     | 0.628009   | 287 | 3   | 167 |
| 1001     | 0.702      | 297 | −31 | 157 | 3039     | 0.627907   | 324 | 62  | 130 |
| 165      | 0.701      | 303 | −22 | 151 | 3420     | 0.627273   | 276 | −14 | 178 |
| 2221     | 0.701      | 328 | 14  | 126 | 3085     | 0.626506   | 260 | −39 | 194 |
| 2579     | 0.700      | 301 | −24 | 153 | 3115     | 0.626223   | 320 | 57  | 134 |
| 4579     | 0.699      | 588 | 387 | −134| 1154     | 0.625541   | 289 | 8   | 165 |
| 1195     | 0.698      | 296 | −30 | 158 | 1153     | 0.625541   | 289 | 8   | 165 |
| 900      | 0.698      | 296 | −30 | 158 | 1169     | 0.625282   | 277 | −11 | 177 |
| 2197     | 0.697      | 355 | 55  | 99  | 161      | 0.625282   | 277 | −11 | 177 |
| 212      | 0.697      | 306 | −15 | 148 | 1140     | 0.625282   | 277 | −11 | 177 |
| 211      | 0.697      | 306 | −15 | 148 | 2588     | 0.625282   | 277 | −11 | 177 |
| 2578     | 0.694      | 300 | −22 | 154 | 4598     | 0.622567   | 287 | 7   | 167 |
| 205      | 0.691      | 318 | 6   | 136 | 848      | 0.622517   | 282 | −1  | 172 |
| Compound | Similarity | SA | SB | SC | Compound | Similarity | SA | SB | SC |
|-----------|------------|----|----|----|-----------|------------|----|----|----|
| 2555      | 0.688      | 296 | −24| 158| 1155      | 0.62203    | 288 | 9  | 166|
| 3079      | 0.687      | 398 | 125| 56 | 3412      | 0.620843   | 280 | −3| 174|
| 4573      | 0.687      | 417 | 153| 37 | 2137      | 0.620536   | 278 | −6| 176|
| 2557      | 0.686      | 308 | −5 | 146| 2407      | 0.620451   | 358 | 123| 96 |
| 4572      | 0.686      | 410 | 144| 44 | 1147      | 0.62        | 279 | −4| 175|
| 3421      | 1          | 311 | 0  | 143| 637       | 0.619048   | 325 | 71 | 129|
| 2202      | 1          | 282 | −42| 172| 2201      | 0.618893   | 380 | 160| 74 |
| 213       | 1          | 311 | 1  | 143| 2199      | 0.618487   | 368 | 141| 86 |
| 2067      | 1          | 449 | 206| 5  | 189       | 0.617849   | 270 | −17| 184|
| 126       | 1          | 294 | −21| 160| 1156      | 0.61753    | 310 | 48 | 144|
| 2070      | 1          | 470 | 239| −16| 2203      | 0.617021   | 261 | −31| 193|
| 1330      | 1          | 293 | −21| 161| 2685      | 0.616725   | 354 | 120| 100|
| 168       | 1          | 301 | −9 | 153| 1992      | 0.616279   | 265 | −24| 189|
| 4575      | 1          | 417 | 163| 37 | 3469      | 0.615       | 246 | −54| 208|
| 1132      | 1          | 387 | 119| 67 | 4879      | 0.614232   | 328 | 80 | 126|
| 3419      | 0.673289   | 305 | −1 | 149| 2         | 0.614191   | 277 | −3 | 177|
| 1133      | 0.673043   | 387 | 121| 67 | 3924      | 0.613333   | 276 | −4 | 178|
| 190       | 0.671772   | 307 | 3  | 147| 713       | 0.612691   | 280 | 3  | 174|
| 1331      | 0.670507   | 291 | −20| 163| 2206      | 0.610132   | 277 | 0  | 177|
| 181       | 0.67033    | 305 | 1  | 149| 1952      | 0.609977   | 269 | −13| 185|
| 1000      | 0.670306   | 307 | 4  | 147| 990       | 0.609865   | 272 | −8 | 182|
| 163       | 0.668161   | 298 | −8 | 156| 3392      | 0.609865   | 272 | −8 | 182|
| 2958      | 0.667814   | 388 | 127| 66 | 166       | 0.609589   | 267 | −16| 187|
| 3923      | 0.667431   | 291 | −18| 163| 3411      | 0.607692   | 553 | 456| −99|
| 926       | 1          | 304 | 3  | 150| 2189      | 0.606762   | 341 | 108| 113|
| 3473      | 1          | 304 | 3  | 150| 3445      | 0.605905   | 472 | 325| −18|
| 2198      | 1          | 375 | 110| 79 | 4580      | 0.60414    | 467 | 319| −13|
| 2065      | 1          | 455 | 231| −1 | 1861      | 0.60414    | 467 | 319| −13|
| 204       | 1          | 290 | −17| 164| 1859      | 0.60414    | 467 | 319| −13|
| 3395      | 1          | 315 | 21 | 139| 3410      | 0.603712   | 553 | 462| −99|
| 215       | 1          | 291 | −13| 163| 4577      | 0.603359   | 467 | 320| −13|
| 4571      | 1          | 405 | 160| 49 | 1642      | 0.602794   | 302 | 47 | 152|
| 169       | 0.658314   | 289 | −15| 165| 1643      | 0.602794   | 302 | 47 | 152|
| 3114      | 0.657505   | 311 | 19 | 143| 1644      | 0.602794   | 302 | 47 | 152|
| 3394      | 0.655602   | 316 | 28 | 138| 2107      | 0.602687   | 314 | 67 | 140|
| 198       | 0.655012   | 281 | −25| 173| 671       | 0.602637   | 320 | 77 | 134|
| 1212      | 0.655012   | 281 | −25| 173| 4578      | 0.602581   | 467 | 321| −13|
| 3124      | 0.653277   | 309 | 19 | 145| 4581      | 0.602581   | 467 | 321| −13|
| 3098      | 0.653277   | 309 | 19 | 145| 1977      | 0.602076   | 348 | 124| 106|
Table 1. Cont.

| Compound | Similarity | SA  | SB | SC  | Compound | Similarity | SA  | SB | SC  |
|----------|------------|-----|----|-----|----------|------------|-----|----|-----|
| 2227     | 0.652268   | 302 | 9  | 152 | 3442     | 0.60177   | 272 | −2 | 182 |
| 2471     | 0.65       | 299 | 6  | 155 | 2194     | 0.601643  | 293 | 33 | 161 |
| 3444     | 0.649886   | 284 | −17| 170 | 2467     | 0.600877  | 542 | 448| −88 |
| 3629     | 0.649874   | 258 | −57| 196 | 2636     | 0.600751  | 480 | 345| −26 |
| 199      | 0.648402   | 284 | −16| 170 | 2635     | 0.600751  | 480 | 345| −26 |
| 1137     | 1          | 419 | 193| 35  | 4750     | 0.600742  | 486 | 355| −32 |
| 1136     | 1          | 419 | 193| 35  | 2496     | 0.600671  | 537 | 440| −83 |
| 1138     | 1          | 419 | 193| 35  | 2467     | 0.600887  | 293 | 33 | 162 |
| 1139     | 1          | 419 | 193| 35  | 3444     | 0.649886  | 284 | −17| 170 |
| 1464     | 0.647208   | 255 | −60| 199 | 2195     | 0.599589  | 292 | 33 | 162 |
| 4574     | 0.646965   | 405 | 172| 49  | 4549     | 0.599567  | 554 | 470| −100|
| 1768     | 1          | 357 | 98 | 97  | 4551     | 0.599341  | 546 | 457| −92 |
| 3754     | 1          | 486 | 298| −32 | 1143     | 0.597802  | 272 | 1  | 182 |
| 2222     | 1          | 285 | −13| 169 | 4128     | 0.597802  | 272 | 1  | 182 |
| 3396     | 1          | 313 | 31 | 141 | 1352     | 0.597802  | 272 | 1  | 182 |
| 155      | 0.645161   | 300 | 11 | 154 | 4601     | 0.597802  | 272 | 1  | 182 |
| 167      | 0.644295   | 288 | −7 | 166 | 2118     | 0.597802  | 272 | 1  | 182 |
| 2888     | 1          | 284 | −13| 170 | 2667     | 0.596899  | 308 | 62 | 146 |
| 4266     | 0.643991   | 284 | −13| 170 | 4486     | 0.596491  | 272 | 2  | 182 |
| 4759     | 0.643392   | 258 | −53| 196 | 3118     | 0.596429  | 334 | 106| 120 |
| 1118     | 0.642132   | 253 | −60| 201 | 3073     | 0.595133  | 269 | −2 | 185 |
| 3113     | 0.642127   | 314 | 35 | 140 | 227      | 0.594771  | 273 | 5  | 181 |
| 3925     | 0.641553   | 281 | −16| 173 | 228      | 0.594771  | 273 | 5  | 181 |
| 2220     | 0.640244   | 315 | 38 | 139 | 3071     | 0.594714  | 270 | 0  | 184 |
| 4899     | 1          | 282 | −13| 172 | 4570     | 0.594714  | 270 | 0  | 184 |
| 279      | 1          | 282 | −13| 172 | 4897     | 0.594714  | 270 | 0  | 184 |
| 1447     | 1          | 282 | −13| 172 | 2684     | 0.594454  | 343 | 123| 111 |
| 1559     | 1          | 282 | −13| 172 | 4485     | 0.594421  | 277 | 12 | 177 |
| 281      | 0.639456   | 282 | −13| 172 | 278      | 0.594298  | 542 | 458| −88 |
| 164      | 0.639269   | 280 | −16| 174 | 4896     | 0.593407  | 270 | 1  | 184 |
| 4468     | 0.639098   | 340 | 78 | 114 | 1117     | 0.593407  | 270 | 1  | 184 |
| 1134     | 0.638066   | 409 | 187| 45  | 1152     | 0.593254  | 299 | 50 | 155 |
| 1135     | 0.638066   | 409 | 187| 45  | 1448     | 0.593148  | 277 | 13 | 177 |
| 2200     | 1          | 376 | 138| 78  | 4129     | 0.593148  | 277 | 13 | 177 |
| 1949     | 0.634033   | 272 | −25| 182 | 1329     | 0.593148  | 277 | 13 | 177 |
| 4592     | 0.633047   | 295 | 12 | 159 |

SA: The number of bits in both TTT and the target. SB: The number of bits in the target but not TTT. SC: The number of bits in TTT but not the target.

3.2. Molecular Similarity

The molecular similarity study differs from the fingerprints study in that the first computes certain descriptors regarding the whole chemical structure of a molecule. The
computed descriptors are topological, steric, electronic, and/or physical properties [50]. On the other hand, the fingerprints study compares the absence or presence of certain 2D atom paths, fragments, or substructures in the chemical structures of reference and test molecules [51].

Employing Discovery studio software, the molecular similarities of the selected 100 natural metabolites were investigated correlating TTT. The employed properties in this study (Figure 1 and Table 2) were partition coefficient (ALog p) [52], molecular weight (M. Wt) [53], H- bond donors (HBA) [54], H- bond acceptors (HBD) [55], rotatable bonds number [56], number of rings along with aromatic rings [57], and minimum distance [58] as well as the molecular fractional polar surface area (MFPSA) [59]. The experiment was adjusted to extract the most similar 40 compounds (Figure 2).

![Figure 1. Molecular similarity analysis of the African metabolites and TTT.](image)

| Compound | ALog p | M. Wt  | HBA | HBD | Rotatable Bonds | Rings | Aromatic Rings | MFPSA | Minimum Distance |
|----------|--------|--------|-----|-----|-----------------|-------|---------------|-------|-----------------|
| 126      | 2.73   | 272.30 | 4   | 2   | 2               | 3     | 2             | 0.22  | 0.731           |
| 165      | 2.84   | 270.28 | 4   | 1   | 2               | 3     | 2             | 0.211 | 0.731           |
| 2579     | 2.88   | 268.26 | 4   | 1   | 2               | 3     | 2             | 0.215 | 0.730           |
| 182      | 2.71   | 256.30 | 3   | 1   | 2               | 3     | 2             | 0.151 | 0.718           |
| 189      | 2.41   | 267.28 | 3   | 1   | 1               | 4     | 3             | 0.216 | 0.696           |
| 2203     | 3.24   | 252.27 | 2   | 2   | 0               | 4     | 3             | 0.244 | 0.661           |
| 204      | 4.18   | 270.32 | 3   | 1   | 2               | 3     | 2             | 0.139 | 0.660           |
| 164      | 3.46   | 272.30 | 4   | 2   | 2               | 3     | 2             | 0.22  | 0.659           |
| 3395     | 3.55   | 298.33 | 4   | 2   | 4               | 3     | 2             | 0.229 | 0.656           |
| 181      | 3.18   | 300.35 | 4   | 1   | 3               | 3     | 2             | 0.153 | 0.652           |
Table 2. Cont.

| Compound | ALog p | M. Wt  | HBA | HBD | Rotatable Bonds | Rings | Aromatic Rings | MFPSA | Minimum Distance |
|----------|--------|--------|-----|-----|----------------|-------|----------------|-------|------------------|
| 2202     | 3.48   | 236.27 | 1   | 1   | 0              | 4     | 3              | 0.167 | 0.649            |
| 2137     | 4.14   | 266.33 | 2   | 2   | 2              | 3     | 2              | 0.145 | 0.619            |
| 3396     | 3.64   | 284.35 | 3   | 2   | 4              | 3     | 2              | 0.173 | 0.587            |
| 212      | 3.21   | 265.26 | 3   | 1   | 1              | 4     | 3              | 0.207 | 0.556            |
| 211      | 3.21   | 265.26 | 3   | 1   | 1              | 4     | 3              | 0.207 | 0.556            |
| 213      | 3.43   | 279.29 | 3   | 0   | 2              | 4     | 3              | 0.148 | 0.545            |
| 2101     | 3.31   | 323.39 | 4   | 2   | 6              | 3     | 3              | 0.21  | 0.545            |
| 1875     | 4.69   | 264.32 | 2   | 2   | 1              | 3     | 3              | 0.147 | 0.502            |
| 3421     | 3.01   | 281.31 | 3   | 1   | 2              | 4     | 3              | 0.157 | 0.493            |
| 3412     | 3.09   | 282.24 | 3   | 3   | 2              | 3     | 2              | 0.264 | 0.843            |
| 2982     | 3.29   | 270.32 | 3   | 1   | 5              | 2     | 2              | 0.204 | 0.822            |
| 166      | 2.63   | 242.23 | 4   | 1   | 2              | 3     | 2              | 0.245 | 0.816            |
| 3323     | 3.14   | 222.24 | 2   | 0   | 1              | 3     | 2              | 0.123 | 0.815            |
| 197      | 3.29   | 262.31 | 1   | 0   | 2              | 4     | 4              | 0.116 | 0.811            |
| 215      | 2.69   | 233.23 | 3   | 0   | 0              | 4     | 3              | 0.154 | 0.809            |
| 2981     | 4.37   | 252.31 | 2   | 1   | 5              | 2     | 2              | 0.139 | 0.807            |
| 198      | 2.32   | 257.28 | 4   | 2   | 3              | 3     | 2              | 0.196 | 0.788            |
| 3469     | 2.46   | 182.22 | 1   | 1   | 0              | 3     | 3              | 0.154 | 0.785            |
| 3444     | 3.04   | 268.26 | 4   | 1   | 1              | 3     | 2              | 0.241 | 0.781            |
| 2578     | 3.20   | 270.28 | 4   | 2   | 4              | 2     | 2              | 0.239 | 0.774            |
| 1330     | 3.20   | 270.28 | 4   | 2   | 4              | 2     | 2              | 0.239 | 0.774            |
| 1331     | 3.20   | 270.28 | 4   | 2   | 4              | 2     | 2              | 0.239 | 0.774            |
| 2195     | 4.00   | 338.35 | 5   | 3   | 3              | 3     | 2              | 0.257 | 0.772            |
| 3040     | 4.83   | 350.41 | 4   | 2   | 4              | 3     | 2              | 0.185 | 0.768            |
| 4598     | 2.86   | 298.29 | 5   | 1   | 3              | 3     | 2              | 0.22  | 0.761            |
| 3518     | 2.99   | 226.27 | 2   | 1   | 1              | 3     | 2              | 0.133 | 0.757            |
| 168      | 2.98   | 251.24 | 3   | 2   | 0              | 4     | 3              | 0.278 | 0.754            |
| 2677     | 3.30   | 265.31 | 3   | 0   | 2              | 3     | 2              | 0.107 | 0.743            |
| 199      | 2.73   | 271.31 | 4   | 1   | 4              | 3     | 2              | 0.14  | 0.741            |
| 1952     | 3.88   | 230.26 | 3   | 2   | 2              | 2     | 2              | 0.205 | 0.739            |
| TTT      | 3.65   | 304.39 | 2   | 2   | 3              | 3     | 3              | 0.171 |               |
Figure 2. The most similar African metabolites to TTT.
3.3. Docking Studies

The forty most structurally similar compounds were subjected to a molecular docking study in the hope of getting an insight into the way they interact with their biomolecular target. The papain-like protease (PLpro) crystal structure PDB ID: 3E9S in complex with the co-crystallized ligand, **TTT**, was adopted for the present study. A docking study was performed using MOE 14.0 software, Montreal, Canada. The calculated ∆G of the tested compounds is cited in Table 3.

Table 3. The calculated ∆G values of the African metabolites and TTT.

| Compound | ∆G  | Compound | ∆G  |
|----------|-----|----------|-----|
| 126      | −10.70 | 2982     | −11.85 |
| 165      | −9.81  | 166      | −7.96  |
| 2579     | −9.17  | 3323     | −10.11 |
| 182      | −12.74 | 197      | −9.72  |
| 189      | −8.65  | 215      | −13.18 |
| 2203     | −8.14  | 2981     | −13.72 |
| 204      | −12.21 | 198      | −8.01  |
| 164      | −13.22 | 3469     | −5.40  |
| 3395     | −7.62  | 3444     | −8.88  |
| 181      | −11.55 | 2578     | −9.51  |
| 2202     | −9.22  | 1330     | −12.20 |
| 2137     | −9.98  | 1331     | −14.09 |
| 3396     | −14.14 | 2195     | −16.52 |
| 212      | −12.39 | 3040     | −14.25 |
| 211      | −10.35 | 4598     | −10.13 |
| 213      | −12.63 | 3518     | −7.54  |
| 2197     | −13.10 | 168      | −16.41 |
| 1875     | −8.65  | 2677     | −9.54  |
| 3421     | −7.76  | 199      | −10.79 |
| 3412     | −14.00 | 1952     | −12.93 |
| TTT      | −9.30  |          |       |

The docking protocol was first validated via the redocking of the co-crystallized ligand (TTT) against the active pocket of SARS papain-like protease (PLpro) active pocket. However, the validation step proved the suitability of the performed protocol for the intended docking study, as demonstrated by the small RMSD (0.51 Å) between the docked pose and the co-crystallized ligand (Figure 3).

**TTT**, the well-known PLpro inhibitor, was used as a reference in the current study. The binding affinity value of **TTT** was −9.30 kcal/mol. **TTT** interacted with the active pocket through the formation of two H-bonds. The amidic NH group of **TTT** formed an H-bond with the carboxylic acid side chain of Asp165, while the amidic carbonyl bound to the nitrogen backbone of Gln270. Additionally, the naphthyl moiety was involved in a hydrophobic interaction with the Pro249 side chain (Figure 4).
Figure 3. Superimposition of the co-crystallized ligand pose (pink) and the docking pose (wheat).

Figure 4. The proposed binding pattern of TTT against the PLpro active site.

Results of the docking study showed that most of the tested compounds have a similar position and orientation inside the SARS PLpro active site. Among them, members 2195, 1952, 2982, and 1330 revealed the greatest binding free energies of docking, which were almost close to the redocked ligand.

The docking simulation of compound 2195 revealed that it has the highest fitting into the enzyme active site with a docking score of $-24.88 \text{ kcal/mol}$. It was stabilized in the active site through the formation of six H-bond interactions and many hydrophobic interactions. The chromenone moiety, via its carbonyl and hydroxyl groups, formed five H-bonds with Tyr269, Gln270, Leu263, and Lys253. On the other side, the p-hydroxyphenyl moiety was involved in an H-bond with Arg167 (Figure 5). The chromenone moiety and the phenyl ring also formed hydrophobic interactions with Tyr269 and Lys158, respectively.

Compound 1952 exhibited a binding mode similar to that of the co-crystallized ligand with the formation of two H-bonds. One H-bond was formed between a hydroxyl group with Asp165. The other H-bond was formed between the oxygen bridge and Gln270 (Figure 6). Additionally, a hydrophobic interaction was formed between one phenyl moiety and Tyr265 of the active site.

A study of the top docking poses of member 2982 (Figure 7) showed that it interacted with the PLpro active site through the formation of three H-bond interactions. The hydroxyphenyl moiety was involved in an H-bonding with Leu163, while the aliphatic hydroxyl...
group formed another H-bond with Asp165. In addition, the carbonyl group formed an H-bond with Gln270.

Figure 5. The proposed binding pattern of compound 2195.

Figure 6. The proposed binding pattern of compound 1952.

Figure 7. The proposed binding pattern of compound 2982.

A study of the top docking poses of member 2982 (Figure 7) showed that it interacted with the PLpro active site through the formation of three H-bond interactions. The hydroxyphenyl moiety was involved in an H-bonding with Leu163, while the aliphatic hydroxyl group formed another H-bond with Asp165. In addition, the carbonyl group formed an H-bond with Gln270.

Figure 7. The proposed binding pattern of compound 2982.
Figure 8 illustrates the proposed binding mode of compound 1330. The two phenolic hydroxyl groups interacted with the active site by two H-bonds with Ala247 and Gln270. Furthermore, the carbonyl group formed an H-bond with Asp165.

3.4. ADMET Studies

The ability of a molecule to be a drug is decided not only by activity but also by acceptable pharmacokinetic properties. ADMET profile describes absorption, distribution, metabolism, excretion, and toxicity. Although the determination of the ADMET profile is available via several medium- and high-throughput in vitro methods, the ability to predict it depending on in silico is available with the advantage of saving time, money, effort, and animal lives [60]. ADMET prediction is an essential step in drug discovery [61].

The computed ADMET descriptors for 17 compounds that displayed correct binding mode and energy, as well as remdesivir as a reference drug, are listed in (Table 4 and Figure 9). Compounds 181, 182, 204, 212, 213, 215, 1952, 2981, 3040, and 3396 were expected to have a high ability to pass BBB and, accordingly, were eliminated. Fortunately, the absorption levels of all compounds were computed as good. Similarly, all of them showed low to good aqueous solubility levels. All compounds were expected to bind to plasma protein with a ratio of more than 90%. Finally, according to these results, compounds Hippacine (164), Naamine D (2197), (+)-Enterofuran (3412), Daphnelone (2982), 4,2′-dihydroxy-4′-methoxychalcone (1330), 2′,5′-dihydroxy-4-methoxychalcone (1331), and wighteone (2195) were favored and subjected to the next toxicity examination.

| Compound | BBB Level a | HIA b | Aq c | CYP2D6 d | PPB e |
|----------|-------------|-------|------|-----------|-------|
| 164      | 2           | 0     | 3    | f         | t     |
| 181      | 1           | 0     | 2    | t         | t     |
| 182      | 1           | 0     | 3    | t         | t     |
| 204      | 1           | 0     | 2    | t         | t     |
| 212      | 1           | 0     | 2    | f         | t     |
| 213      | 1           | 0     | 2    | f         | t     |
| 215      | 1           | 0     | 2    | t         | t     |
| 1330     | 2           | 0     | 3    | f         | t     |
Table 4. Cont.

| Compound | BBB Level a | HIA b | Aq c | CYP2D6 d | PPB e |
|----------|-------------|-------|------|-----------|-------|
| 1331     | 2           | 0     | 3    | f         | t     |
| 1952     | 1           | 0     | 3    | f         | t     |
| 2195     | 2           | 0     | 2    | t         | t     |
| 2197     | 2           | 0     | 2    | t         | t     |
| 2981     | 1           | 0     | 2    | f         | t     |
| 2982     | 2           | 0     | 3    | t         | t     |
| 3040     | 1           | 0     | 2    | f         | t     |
| 3396     | 1           | 0     | 3    | f         | t     |
| 3412     | 2           | 0     | 3    | f         | t     |

Remdesivir 4 3 3 f f

a BBB, Ability to pass the blood-brain barrier, 1 is high, 2 is medium, 3 is low, 4 is very low. b HIA, human intestinal absorption level, 0 is good, 1 is moderate, 2 is poor, and 3 is very poor. c Aq. Aqueous solubility level, 0 is extremely low, 1 is very low, 2 is low, 3 is good, and 4 is optimal. d CYP2D6, inhibition of CYP2D6 enzyme, t is an inhibitor, f is a non-inhibitor. e PPB, f means less than 90%, t means more than 90%.

Figure 9. ADMET profile of the African metabolites and the reference.

3.5. Toxicity Studies

The prediction of toxicity of a molecule depending on computer software (in silico) has been employed effectively to select drug leads in the field of drug design, as in vitro and in vivo methods are usually limited by lack of time, budget, and ethical restrictions [62,63].

The toxicity of 7 compounds that displayed good ADMET profiles was predicted in silico using Discovery Studio software concerning 7 different models. The employed models...
are FDA rat carcinogenicity [64,65], carcinogenic potency median toxic dose, (TD$_{50}$) [66], rat maximum tolerated dose (MTD) [67,68], rat oral LD$_{50}$ [69], rat chronic lowest-observed-adverse-effect level (LOAEL) [70,71], and ocular and skin irritancy [72]. As shown in Table 5, compounds 2982 and 3412 were proposed as carcinogenic. In consequence, both were refused. Also, all compounds, excluding 2197, are expected to have TD$_{50}$ and TD$_{50}$ values more than the reference. Thus, 2197 was excluded too. All compounds were computed to have LOAEL values more than the reference and to be non-irritant in the skin model. On the other hand, all compounds except 1330 showed different degrees of ocular irritancy.

Table 5. Toxicity properties of filtered African metabolites and the reference.

| Compound                  | FDA Rat Carcinogenicity | TD$_{50}$ (Rat) $^a$ | MTD $^b$ | Rat Oral LD$_{50}$ $^b$ | LOAEL $^b$ | Ocular Irritancy | Skin Irritancy |
|---------------------------|-------------------------|----------------------|----------|-------------------------|------------|-----------------|---------------|
| Hippacine (164)           | Not carcinogenic        | 63.019               | 0.285    | 0.441                   | 0.052      | Mild            | None          |
| Naamine D (2197)          | Not carcinogenic        | 4.022                | 0.086    | 2.440                   | 0.015      | Moderate        | None          |
| (±)-Enterofuran (3412)    | Carcinogenic            | 87.484               | 0.690    | 2.483                   | 0.089      | Severe          | None          |
| Daphnelone (2982)         | Carcinogenic            | 184.723              | 0.829    | 0.646                   | 0.173      | Severe          | None          |
| 4,2′-dihydroxy-4′-methoxychalcone (1330) | Not carcinogenic | 259.532              | 0.320    | 1.010                   | 0.060      | None            | None          |
| 2′,5′-dihydroxy-4-methoxychalcone (1331) | Not carcinogenic | 259.532              | 0.320    | 1.010                   | 0.060      | Mild            | None          |
| Wighteone (2195)          | Not carcinogenic        | 42.573               | 0.525    | 0.962                   | 0.053      | Severe          | None          |
| Remdesivir                | Not carcinogenic        | 9.246                | 0.235    | 0.309                   | 0.004      | Mild            | Mild          |

$^a$ Unit: mg kg$^{-1}$ day$^{-1}$. $^b$ Unit: g kg$^{-1}$.

The acquired results privilege compounds Hippacine (164), 4,2′-dihydroxy-4′-methoxychalcone (1330), 2′,5′-dihydroxy-4-methoxychalcone (1331), and wighteone (2195) as the most convenient inhibitors against the target enzyme. Among the selected compounds, wighteone displayed the most favorable docking score and energy. Wighteone is an isoflavonoid that has been isolated from the bark of a South African *Erythrina* species showing promising antibacterial effects [73]. It was also isolated from several *Maclura* species before [74,75]. The antiviral activity of wighteone against HIV has been reported in vitro [76], and its in silico potentiality against HIV-1 protease enzyme with a binding affinity of $-8.7$ Kcal/mol [77].

3.6. Molecular Dynamics (MD) Simulations

Although molecular docking can predict the correct binding poses of a molecule inside the active site of a certain protein, it has a major drawback in that it considers the proteins rigid, and thus doesn’t allow the protein to adjust its conformation during the docking process [78]. On the other hand, MD simulations can efficiently predict how every single atom in a specific protein will move over a specific time, depending on a physical model of the interatomic interactions [79]. Correspondingly, MD simulations have been successfully utilized to examine the conformation changes in protein-ligand interactions and protein dynamics and folding [80]. MD simulation is an effective and accurate in silico technique that can describe the binding mode, stability, and flexibility of a certain receptor and a specific ligand for a determined time [81].

Molecular dynamics (MD) simulations were carried out to mimic the dynamic nature of PLpro-wighteone interaction under physiological conditions and to investigate the stability of binding complex simulation for 100 ns.
RMSD and RMSF Analysis

The binding of a certain ligand in a specific protein causes notable changes in the structure [82]. Consequently, the root mean square deviation (RMSD) parameter was investigated to explore whether the structure of the PLpro-wighteone complex is stable and near the experimental structure. Figure 10 shows that the PLpro-wighteone complex exhibited a good RMSD value along with 100 ns MD; the PLpro showed an RMSD value of 2.5 Å too, while the complex exhibited an average RMSD value of 3.5 Å, below the acceptable range of 4 Å. After 60 ns, no dramatic increment in the RMSD values was noticed and the complex system reached equilibrium.

![Graph showing RMSD values during MD runs](image)

**Figure 10.** RMSD value during MD runs. (Red: (wighteone), blue: (PLpro), black: (PLpro-wighteone complex)).

Root mean square fluctuation (RMSF) was utilized to describe the flexibility differences among wighteone, PLpro, and their complex during the MD simulation for 100 ns. Increasing RMSF value denotes a higher degree of flexibility, while the low value is related to limited movement during the MD simulation. To investigate the average fluctuation of PLpro during the MD study, the RMSF of PLpro upon the binding of wighteone was plotted as a function of residue number (Figure 11). RMSF plot indicated that the residual fluctuation of PLpro was minimized upon binding of wighteone. This result indicates that PLpro residues were more rigid in the presence of wighteone because of binding to wighteone.
The radius of gyration ($R_g$) is an essential parameter that gives a clear insight into the protein stability in terms of volume change. $R_g$ is defined as the RMSD of the mass-weighted of a group of atoms from their common mass center [83,84]. Accordingly, the analysis of $R_g$ of PLpro during the MD simulation will describe its overall dimensions. The average $R_g$ values were found to suggest the tight packing of PLpro in its native state and when bound to wighteone. PLpro-wighteone complex reached a stable conformation with the radius of gyration fluctuating around 24.4 Å (Figure 12).

![Figure 11. RMSF of PLpro in the MD run.](image1)

The solvent-accessible surface area (SASA) is the surface area of the protein which can be accessible to a solvent [85]. The evaluation of SASA provides information about the conformational changes that happen in a protein because of ligand binding. The average SASA values for PLpro were monitored during 100 ns MD simulations. As shown in Figure 13, there were no major changes in the values of SASA of PLpro due to wighteone binding.

![Figure 12. The radius of gyration of PLpro in the MD run.](image2)
shown in Figure 13, there were no major changes in the values of SASA of PLpro due to wighteone binding.

Figure 13. SASA of PLpro in the MD run.

4. Discussion

The recent advancement in software enabled computational chemistry to perfectly describe the physical and chemical properties of a compound in addition to its potential to interact with a particular protein.

Accordingly, several researchers utilized computational chemistry to identify potential inhibitors against SARS-CoV-2 using different approaches. Exploring the potentialities of FDA-approved antivirus drugs against SARS-CoV-2 was one of the first computational approaches. For instance, the computational potentialities of remdesivir, the FDA-approved anti-ebola, and respiratory syncytial viruses against SARS-CoV-2 main protease were investigated [86]. The same approach was applied to lopinavir/ritonavir [87] and ribavirin [88] targeting SARS-CoV-2 3-chymotrypsin-like protease. One of the employed approaches was the computational-based drug repurposing of non-antiviral FDA-approved drugs such as lurasidone (anti-schizophrenia) against SARS-CoV-2 3CL hydrolase and protease enzymes [89], aclarubicin [90], and selinexor [91], FDA-approved anti-cancers that exhibited computational activities against SARS-CoV-2 main protease.

Our team employed computational chemistry to develop a multiphase in silico technique to discover the most appropriate natural inhibitor via large sets of molecules against a specific enzyme of COVID-19. Within 310 natural antiviral metabolites, the most effective inhibitor against SARS-CoV-2 nsp10 [92], main protease [93,94], and papain-like protease [95] were predicted. Also, within 3009 FDA approved drugs, the most potent inhibitors against SARS-CoV-2 nsp16-nsp10 2'-o-Methyltransferase Complex [96] and SARS-CoV-2 RNA-Dependent RNA Polymerase [97] were anticipated. The SARS-CoV-2 Helicase potential natural inhibitors were expected among 5956 compounds of traditional Chinese medicine also [98]. Further, the most active semisynthetic COVID-19 papain-like protease inhibitor was discovered amidst 69 molecules [99].

Unfortunately, at the current time, we don’t have access to investigate the experimental inhibitory effects of the pointed 4 metabolites (Hippacine, 4,2'-dihydroxy-4'-methoxychalcone,
2′,5′-dihydroxy-4-methoxychalcone, and wighteone) among 4924 African natural metabolites against SARS-CoV-2. However, we presented those 4 metabolites for all scientists worldwide to conduct further in vitro and in vivo studies. The binding potentialities of those metabolites against the SARS-CoV-2 papain-like protease were confirmed through 4 stages of in silico experiments:

Stage I: Selection of the most similar metabolites to the co-crystallized ligand (TTT) of SARS-CoV-2 papain-like protease (PDB ID: 3E9S) (fingertips and molecular similarity studies). This stage selected the most similar 40 metabolites to the co-crystallized ligand;

Stage II: Evaluation and filtration according to the binding against papain-like protease by molecular docking to select 17 metabolites that showed correct binding;

Stage III: Evaluation and the filtration according to drug-likeness by ADMET and toxicity studies to point out the safest and most drug-like 4 metabolites;

Stage IV: Confirmation of the binding against papain-like protease by MD simulations to confirm the binding, conformational and energetic changes that combine the binding process. SARS-CoV-2 papain-like protease (PLpro, PDB ID: 3E9S). A multi-phased in silico approach was employed to select the most similar metabolites to the co-crystallized ligand (TTT).

5. Conclusions

Four metabolites, Hippacine (164), 4,2′-dihydroxy-4′-methoxychalcone (1330), 2′,5′-dihydroxy-4-methoxychalcone (1331), and Wighteone (2195), were selected through 4924 African natural products as the most potent inhibitor against Sars-Cov-2 papain-like protease. The selection is based on multiphase (six experiments) in silico studies. The structural fingerprint study against the co-crystallized ligand (TTT) of SARS-CoV-2 papain-like protease (PDB ID: 3E9S), chemical structural similarity study, molecular docking studies against SARS-CoV-2 papain-like protease (PDB ID: 3E9S), ADMET, and toxicity profiles. Wighteone (2195), the metabolite with the best docking score, was subjected to the molecular dynamics simulation (MD) at 100 ns confirming the binding of wighteone against the target enzyme. We present these interesting results for all scientists worldwide to conduct further in vitro and in vivo studies concerning these promising natural metabolites.

Supplementary Materials: The detailed methodology of this manuscript can be downloaded at: https://www.mdpi.com/article/10.3390/metabo12111122/s1, (Similarity, fingerprints, docking, and MD simulations).

Author Contributions: Project administration, A.M.M. and I.H.E.; Supervision, A.M.M. and I.H.E.; Funding acquisition, E.B.E., A.A.A. and B.A.A.; Methodology, M.M.K. and A.-A.M.M.E.-A.; Validation, I.H.E.; Writing—original draft, A.M.M.; Writing—review and editing, E.B.E., A.A.A., A.M.M., B.A.A. and I.H.E. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNURSP2022R142), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available in the main article and the supplementary materials.

Acknowledgments: The authors extend their appreciation to the Research Center at AlMaarefa University for funding this work.

Conflicts of Interest: No conflict of interest regarding this paper to be declared.

References
1. WHO. WHO Coronavirus (COVID-19) Dashboard. Available online: https://covid19.who.int/ (accessed on 12 November 2022).
2. Engel, T. Basic overview of chemoinformatics. J. Chem. Inf. Model. 2006, 46, 2267–2277. [CrossRef] [PubMed]
27. Yousef, R.; Sakr, H.; Eissa, I.; Mehany, A.; Metwaly, A.; ElHendawy, M.A.; Radwan, M.; ElSohly, M.A.; Abulkhair, H.S.; El-Adl, K. New quinoxaline-2 (1H)-ones as potential VEGFR-2 inhibitors: Design, synthesis, molecular docking, ADMET profile and anti-proliferative evaluations. New J. Chem. 2021, 45, 16949–16964. [CrossRef]

28. Amer, I.H.; Alothabi, S.H.; Trawneh, A.H.; Metwaly, A.M.; Eissa, I.H. Anticancer activity, spectroscopic and molecular docking of some new synthesized sugar hydrazones, Arylidene and α-Aminophosphonate derivatives. Arab. J. Chem. 2021, 14, 103348. [CrossRef]

29. Alesawy, M.S.; Al-Karmalawy, A.A.; Elkaeedy, E.B.; Alswah, M.; Belal, A.; Taghour, M.S.; Eissa, I.H. Design and discovery of new 1,2,4-triazolo[4,3-c] quinazolines as potential DNA intercalators and topoisomerase II inhibitors. Arch. Der Pharm. 2021, 354, 2000237. [CrossRef]

30. Jo, S.; Kim, T.; Iyer, V.G.; Im, W. CHARMM-GUI: A web-based graphical user interface for CHARMM. J. Chem. Comput. Sci. 2008, 29, 1859–1865. [CrossRef] [PubMed]

31. Brooks, B.R.; Brooks, C.L., III; Mackerell, A.D., Jr.; Nilsson, L.; Pettrella, R.J.; Roux, B.; Won, Y.; Archontis, G.; Bartels, C.; Boresch, S.; et al. CHARMM: The biomolecular simulation program. J. Comput. Chem. 2009, 30, 1545–1614. [CrossRef] [PubMed]

32. Lee, J.; Cheng, X.; Swails, J.M.; Yeom, M.S.; Eastman, P.K.; Lemkul, J.A.; Wei, S.; Buckner, J.; Jeong, J.C.; Qi, Y.; et al. CHARMM-GUI Input Generator for NAMD, GROMACS, AMBER, OpenMM, and CHARMM/OpenMM Simulations Using the CHARMM36 Additive Force Field. J. Chem. Theory Comput. 2016, 12, 405–413. [CrossRef] [PubMed]

33. Best, R.B.; Zhu, X.; Shim, J.; Lopes, P.E.; Mittal, J.; Feig, M.; Mackerell, A.D., Jr. Optimization of the additive CHARMM all-atom force field targeting improved sampling of the backbone phi, psi and side-chain chi(1) and chi(2) dihedral angles. J. Chem. Theory Comput. 2012, 8, 3257–3273. [CrossRef]

34. Phillips, J.C.; Braun, R.; Wang, W.; Gumbart, J.; Tajkhorshid, E.; Villa, E.; Chipot, C.; Skeel, R.D.; Kale, L.; Schulten, K. Scalable molecular dynamics with NAMD. J. Comput. Chem. 2005, 26, 1878–1882. [CrossRef] [PubMed]

35. Wawer, M.; Peltsan, L.; Weskamp, N.; Teckentrup, A.; Bajorath, J. Structure–activity relationship anatomy by network-like similarity graphs and local structure–activity relationship indices. J. Med. Chem. 2008, 51, 6075–6084. [CrossRef] [PubMed]

36. Farhadi, F.; Khameneh, B.; Iranshahi, M.; Iranshahi, M. Antibacterial activity of flavonoids and their structure–activity relationship: An update review. Phytother. Res. 2019, 33, 13–40. [CrossRef] [PubMed]

37. Burke, B.J. Developments in Molecular Shape Analysis to Establish Spatial Similarity among Flexible Molecules; University of Illinois at Chicago, Health Sciences Center: Chicago, IL, USA, 1993.

38. Willett, P. Similarity-based virtual screening using 2D fingerprints. Drug Discov. Today 2006, 11, 1046–1053. [CrossRef] [PubMed]

39. Briem, H.; Kuntz, I.D. Molecular similarity based on DOCK-generated fingerprints. J. Med. Chem. 1996, 39, 3401–3408. [CrossRef] [PubMed]

40. Willett, P. Similarity searching using 2D structural fingerprints. Cheminformatics. Comput. Chem. Biol. 2010, 672, 133–158.

41. Spackman, M.A.; McKinnon, J.J. Fingerprinting intermolecular interactions in molecular crystals. CrystEngComm 2002, 4, 378–392. [CrossRef]

42. Chu, H.; He, Q.-X.; Wang, J.; Hu, Y.; Wang, Y.-Q.; Lin, Z.-H. In silico design of novel benzohydroxamate-based compounds as inhibitors of histone deacetylase 6 based on 3D-QSAR, molecular docking, and molecular dynamics simulations. New J. Chem. 2020, 44, 21201–21210. [CrossRef]

43. Ieritano, C.; Campbell, J.L.; Hopkins, W.S. Predicting differential ion mobility behaviour in silico using machine learning. Analyst 2021, 146, 4737–4743. [CrossRef]

44. Alesawy, M.S.; Al-Karmalawy, A.A.; Elkaeedy, E.B.; Alswah, M.; Belal, A.; Taghour, M.S.; Eissa, I.H. Design and discovery of new 1,2,4-triazolo[4,3-c] quinazolines as potential DNA intercalators and topoisomerase II inhibitors. Arch. Der Pharm. 2021, 354, 2000237. [CrossRef] [PubMed]

45. Heikamp, K.; Bajorath, J. How do 2D fingerprints detect structurally diverse active compounds? Revealing compound subset-specific fingerprint features through systematic selection. J. Chem. Inf. Model. 2011, 51, 2254–2265. [CrossRef] [PubMed]

46. Opo, F.A.D.M.; Rahman, M.M.; Ahammad, F.; Ahmed, I.; Bhiuyan, M.A.; Asiri, A.M. Structure based pharmacophore modeling, virtual screening, molecular docking and ADMET approaches for identification of natural anti-cancer agents targeting XIAP protein. Sci. Rep. 2021, 11, 4049. [CrossRef]

47. Duan, J.; Dixon, S.L.; Lowrie, J.F.; Sherman, W. Analysis and comparison of 2D fingerprints: Insights into database screening performance using eight fingerprint methods. J. Mol. Graph. Model. 2010, 29, 157–170. [CrossRef] [PubMed]

48. Sastry, M.; Lowrie, J.F.; Dixon, S.L.; Sherman, W. Large-scale systematic analysis of 2D fingerprint methods and parameters to improve virtual screening enrichments. J. Chem. Inf. Model. 2010, 50, 771–784. [CrossRef]

49. Kogej, T.; Engkvist, O.; Blomberg, N.; Muresan, S. Multifingerprint based similarity searches for targeted class compound selection. J. Chem. Inf. Model. 2006, 46, 1201–1213. [CrossRef]

50. Maggiora, G.; Vogt, M.; Stumpfè, D.; Bajorath, J. Molecular similarity in medicinal chemistry: Miniperspective. J. Med. Chem. 2014, 57, 3186–3204. [CrossRef]

51. Muegge, I.; Mukherjee, P. An overview of molecular fingerprint similarity search in virtual screening. Expert Opin. Drug Discov. 2016, 11, 137–148. [CrossRef]

52. Turchi, M.; Cai, Q.; Lian, G. An evaluation of in-silico methods for predicting solute partition in multiphase complex fluids—A case study of octanol/water partition coefficient. Chem. Eng. Sci. 2019, 197, 150–158. [CrossRef]

53. Sullivan, K.M.; Enoch, S.J.; Ezendam, J.; Sewald, K.; Roggen, E.L.; Cochrane, S. An adverse outcome pathway for sensitization of the respiratory tract by low-molecular-weight chemicals: Building evidence to support the utility of in vitro and in silico methods in a regulatory context. Appl. Vitr. Toxicol. 2017, 3, 213–226. [CrossRef]
54. Altamash, T.; Amhamed, A.; Aparicio, S.; Atilhan, M. Effect of hydrogen bond donors and acceptors on CO₂ absorption by deep eutectic solvents. *Processes* 2020, 8, 1533. [CrossRef]

55. Wan, Y.; Tian, Y.; Wang, W.; Gu, S.; Ju, X.; Liu, G. In silico studies of diarylpyridine derivatives as novel HIV-1 NNRTIs using docking-based 3D-QSAR, molecular dynamics, and pharmacophore modeling approaches. *RSC Adv.* 2018, 8, 40529–40543. [CrossRef] [PubMed]

56. Escamilla-Gutiérrez, A.; Ribas-Aparicio, R.M.; Córdova-Espinoza, M.G.; Castelán-Vega, J.A. In silico strategies for modeling RNA aptamers and predicting binding sites of their molecular targets. *Nucleosides Nucleotides Nucleic Acids* 2021, 40, 798–807. [CrossRef] [PubMed]

57. Goodrnan, G.; Wilson, R. Comparison of the dependence of the TD50 on maximum tolerated dose for mutagens and nonmutagens. *In Silico Methods for Predicting Drug Toxicity*; Springer: Berlin/Heidelberg, Germany, 2018; pp. 11–19.

58. Jain, A.N. Morphological similarity: A 3D molecular similarity method correlated with protein-ligand recognition. *J. Comput.-Aided Mol. Des.* 2000, 14, 199–213. [CrossRef] [PubMed]

59. Zhang, H.; Ren, J.-X.; Ma, J.-X.; Ding, L. Development of an in silico prediction model for chemical-induced urinary tract toxicity by using naïve Bayes classifier. *Mol. Divers.* 2019, 23, 381–392. [CrossRef]

60. Norinder, U.; Bergström, C.A. Prediction of ADMET properties. *ChemMedChem Chem. Enabling Drug Discov.* 2006, 1, 920–937.

61. Ferreira, L.L.; Andricopulo, A.D. ADMET modeling approaches in drug discovery. *Drug Discov. Today* 2019, 24, 1157–1165. [CrossRef]

62. Idakwo, G.; Luttrell, J.; Chen, M.; Hong, H.; Zhou, Z.; Gong, P.; Zhang, C. A review on machine learning methods for in silico toxicity prediction. *J. Environ. Sci. Health Part C* 2018, 36, 169–191. [CrossRef]

63. Raies, A.B.; Bajic, V.B. In silico toxicology: Computational methods for the prediction of chemical toxicity. *Wiley Interdiscip. Rev. Comput. Mol. Sci.* 2016, 6, 147–172. [CrossRef]

64. Xia, X.; Maliski, E.G.; Gallant, P.; Rogers, D. Classification of kinase inhibitors using a Bayesian model. *J. Med. Chem.* 2004, 47, 4463–4470. [CrossRef]

65. BIVIA QSAR, ADMET and Predictive Toxicology. Available online: https://www.3dsbiovia.com/products/collaborative-science/biovia-discovery-studio/qsar-admet-and-predictive-toxicology.html (accessed on 18 August 2022).

66. Venkatapathy, R.; Wang, N.C.Y.; Martin, T.M.; Harten, P.F.; Young, D. Structure–Activity Relationships for Carcinogenic Potential. *Gen. Appl. Syst. Toxicol.* 2009, 23, 800–802. [CrossRef]

67. Wilhelmus, K.R. The Draize eye test. *Surv. Ophthalmol.* 2001, 45, 493–515. [CrossRef]

68. Venkatapathy, R.; Moudgal, C.J.; Bruce, R.M. Assessment of the oral rat chronic lowest observed adverse effect level model in TOPKAT, a QSAR software package for toxicity prediction. *J. Chem. Inf. Comput. Sci.* 2004, 44, 1623–1629. [CrossRef] [PubMed]

69. Pillay, C.C.; Jäger, A.K.; Mulholland, D.A.; Van Staden, J. Cyclooxygenase inhibiting and anti-bacterial activities of South African Metabolites 2022, 12, 1122

70. Oyama, S.D.O.; Souza, L.A.D.; Baldoiu, D.C.; Sarragiotto, M.H.; Silva, A.A. Preynalyzed flavonoids from Maclura tinctoria fruits. *Química Nova* 2013, 36, 800–802. [CrossRef]

71. Delle Monache, G.; De Rosa, M.C.; Scurria, R.; Vitali, A.; Cuteri, M.; Monaco, B.; Pasqua, G.; Botta, B. Comparison between metabolite productions in cell culture and in whole plant of Maclura pomifera. *Phytochemistry* 1995, 39, 575–580. [CrossRef]

72. Akhtar, A.; Hussain, W.; Rasool, N. Probing the pharmacological binding properties, and reactivity of selective pychochemicals as potential HIV-1 protease inhibitors. *Univ. Sci. 2019, 24, 441–464. [CrossRef]

73. Sousa, S.F.; Fernandes, P.A.; Ramos, M.J. Protein–ligand docking: Current status and future challenges. *Proteins Struct. Func. Bioinform.* 2006, 65, 15–26. [CrossRef]

74. Hollingsworth, S.A.; Dror, R.O. Molecular dynamics simulation for all. *Neuron* 2018, 99, 1129–1143. [CrossRef]

75. Liu, X.; Shi, D.; Zhou, S.; Liu, H.; Liu, H.; Yao, X. Molecular dynamics simulations and novel drug discovery. *Expert Opin. Drug Discov.* 2018, 13, 23–37. [CrossRef] [PubMed]

76. Hansson, T.; Oostenbrink, C.; van Gunsteren, W. Molecular dynamics simulations. *Curr. Opin. Struct. Biol.* 2002, 12, 190–196. [CrossRef]

77. Kuzmanic, A.; Zagrovic, B. Determination of ensemble-average pairwise root mean-square deviation from experimental B-factors. *Biophys. J.* 2010, 98, 861–871. [CrossRef]

78. Liu, P.; Lu, J.; Yu, H.; Ren, N.; Lockwood, F.E.; Wang, Q.J. Lubricant shear thinning behavior correlated with variation of radius of gyration via molecular dynamics simulations. *J. Chem. Phys.* 2017, 147, 084904. [CrossRef]
84. Kumar, K.; Anbarasu, A.; Ramaiah, S. Molecular docking and molecular dynamics studies on β-lactamases and penicillin binding proteins. *Mol. Biosyst.* 2014, 10, 891–900. [CrossRef] [PubMed]

85. Mitternacht, S. FreeSASA: An open source C library for solvent accessible surface area calculations. *F1000Research* 2016, 5, 189. [CrossRef] [PubMed]

86. Naik, V.R.; Munikumar, M.; Ramakrishna, U.; Srujana, M.; Goudar, G.; Naresh, P.; Kumar, B.N.; Hemalatha, R.J. Dynamics, Remdesivir (GS-5734) as a therapeutic option of 2019-nCoV main protease–in silico approach. *J. Biolom. Struct. Dyn.* 2021, 39, 4701–4714. [CrossRef]

87. Magro, P.; Zanella, I.; Pescarolo, M.; Castelli, F.; Quiros-Roldan, E.J.B.J. Lopinavir/ritonavir: Repurposing an old drug for HIV infection in COVID-19 treatment. *Biomed. J.* 2021, 44, 43–53. [CrossRef]

88. Alexpandi, R.; De Mesquita, J.G.; Pandian, S.K.; Ravi, A.V. Quinolines-based SARS-CoV-2 3CLpro and RdRp inhibitors and Spike-RBD-ACE2 inhibitor for drug-repurposing against COVID-19: An in silico analysis. *Front. Microbiol.* 2020, 11, 1796. [CrossRef]

89. Elmezayen, A.D.; Al-Obaidi, A.; Sahin, A.T.; Yelekçi, K. Dynamics, Drug repurposing for coronavirus (COVID-19): In silico screening of known drugs against coronavirus 3CL hydrolase and protease enzymes. *J. Biomol. Struct. Dyn.* 2021, 39, 2980–2992. [CrossRef]

90. Keretsu, S.; Bhujbal, S.P.; Cho, S.J. Rational approach toward COVID-19 main protease inhibitors via molecular docking, molecular dynamics simulation and free energy calculation. *Sci. Rep.* 2020, 10, 17716. [CrossRef] [PubMed]

91. Kouli, S.; Jani, V.; Uppuladinne, M.; Sonavane, U.; Nath, A.K.; Darbaji, H.; Joshi, R. Dynamics, Drug repurposing studies targeting SARS-CoV-2: An ensemble docking approach on drug target 3C-like protease (3CLpro). *J. Biomol. Struct. Dyn.* 2021, 39, 5735–5755. [CrossRef] [PubMed]

92. Eissa, I.H.; Khalifa, M.M.; Elkaeed, E.B.; Hafez, E.E.; Alsfouk, A.A.; Metwaly, A.M. In Silico Exploration of Potential Natural Inhibitors against SARS-CoV-2 nsp10. *Molecules* 2021, 26, 6151. [CrossRef]

93. Elkaeed, E.B.; Youssef, F.S.; Eissa, I.H.; Eldady, H.; Alsfouk, A.A.; Ashour, M.L.; El Hassab, M.A.; Abou-Seri, S.M.; Metwaly, A.M. Multi-Step In Silico Discovery of Natural Drugs against COVID-19 Targeting Main Protease. *Int. J. Mol. Sci.* 2022, 23, 6912. [CrossRef]

94. Elkaeed, E.B.; Eissa, I.H.; Eldady, H.; Abdelalim, A.; Alqaisi, A.M.; Alsfouk, A.A.; Elwan, A.; Metwaly, A.M. A Multistage In Silico Study of Natural Potential Inhibitors Targeting SARS-CoV-2 Main Protease. *Int. J. Mol. Sci.* 2022, 23, 8407. [CrossRef] [PubMed]

95. Elkaeed, E.B.; Metwaly, A.M.; Alesawy, M.S.; Saleh, A.M.; Alsfouk, A.A.; Eissa, I.H. Discovery of Potential SARS-CoV-2 Papain-like Protease Natural Inhibitors Employing a Multi-Phase In Silico Approach. *Life* 2022, 12, 1407. [CrossRef]

96. Elkaeed, E.B.; Eissa, I.H.; Alesawy, M.S.; Saleh, A.M.; Alkaeed, E.B.; Alsfouk, A.A.; El-Attar, A.-A.-M.M.; Metwaly, A.M. Ligand and Structure-Based In Silico Determination of the Most Promising SARS-CoV-2 nsp16-nsp10 2’-O-Methyltransferase Complex Inhibitors among 3009 FDA Approved Drugs. *Molecules* 2022, 27, 2287.

97. Elkaeed, E.B.; Eldady, H.; Belal, A.; Alsfouk, B.A.; Ibrahim, T.H.; Abdelmoaty, M.; Afra, R.K.; Metwaly, A.M.; Eissa, I.H. Multi-Phase In Silico Discovery of Potential SARS-CoV-2 RNA-Dependent RNA Polymerase Inhibitors among 3009 Clinical and FDA-Approved Related Drugs. *Processes* 2022, 10, 530. [CrossRef]

98. Metwaly, A.M.; Elwan, A.; El-Attar, A.-A.-M.M.; Al-Rashood, S.T.; Eissa, I.H. Structure-Based Virtual Screening, Docking, ADMET, Molecular Dynamics, and MM-PBSA Calculations for the Discovery of Potential Natural SARS-CoV-2 Helicase Inhibitors from the Traditional Chinese Medicine. *J. Chem.* 2022, 2022, 7270094. [CrossRef]

99. Alesawy, M.S.; Elkaeed, E.B.; Alsfouk, A.A.; Metwaly, A.M.; Eissa, I. In Silico Screening of Semi-Synthesized Compounds as Potential Inhibitors for SARS-CoV-2 Papain-Like Protease: Pharmacophoric Features, Molecular Docking, ADMET, Toxicity and DFT Studies. *Molecules* 2021, 26, 6593. [CrossRef]