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Virtual screening of a focused small molecule library from the ZINC database to identify potential hits and leads against the CagA protein of *Helicobacter pylori*

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***Helicobacter pylori* infections are on the rise due to antibiotic-resistance so new classes of drugs and antibiotics are urgently needed. The cytotoxin-associated gene A (CagA) protein of *H. pylori* is one of the critical virulence factors for its survival in the challenging acidic environment of the host. In this study we aimed to target the CagA protein and identify small molecules that can bind and inhibit the function of CagA thus interfering with its function. CagA protein was used as a docking receptor and a focused library of small molecules (MA-series) from the ZINC database were chosen as ligands for virtual screening. Swiss Dock was used for the molecular docking of the ligands and their binding affinities were used as docking scores to rank them. Our results show that MA01027 has the highest binding affinity (-11.5 kcal/mol.) among the library and hence it was chosen as the lead molecule for further evaluation. MA01027 will be synthesized and evaluated in the future.**

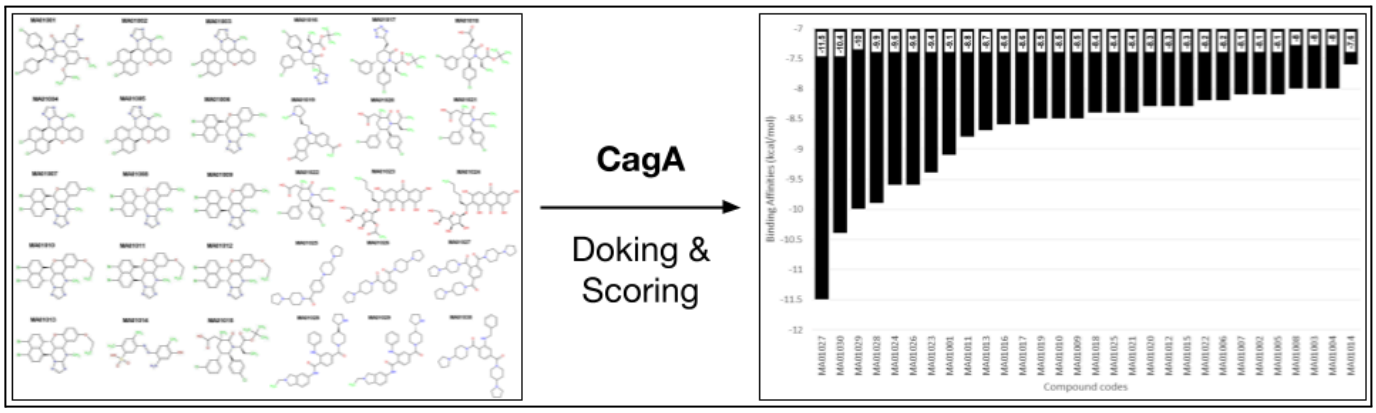

Figure 1. Overall process of virtual screening and scoring of compounds from the ZINC database against *H. pylori* CagA protein.

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Gastric ulcers and gastritis are on the rise in recent years primarily due to the *Helicobacter pylori* infections [1]. *H. pylori* infections affect almost half the world population irrespective of eating habits and ethnic backgrounds [2]. Normally *H. pylori* infections are treated with general antibiotics either with or without proton-pump inhibitors to reduce the infection and ease the ulceration in the acidic environment [3, 4]. The cytotoxin-associated gene A (CagA) encoded protein of *H. pylori* acts as a virulence factor that helps in hijacking the host gastric epithelial cells [5]. CagA is known to escalate the *H. pylori* infections to gastric cancers in many cases [6, 7]. However, currently there are no pharmacological interventions to encounter the CagA protein that can potentially control not only the *H. pylori* infections but also prevent gastric cancers [8]. Additionally, development of antibiotic-resistance is making the situation even worse [9]. The combination of lack of direct pharmacological interventions and increasing antibiotic-resistance calls for new classes of drugs that are specific to the *H. pylori* treatment. In this study, we used the CagA protein as a drug target and virtually screened a focused library of small molecules from the ZINC database [10]. Swiss Dock [11] protocol was used to dock the small molecules one at a time and rank them according to their binding affinities (docking scores).

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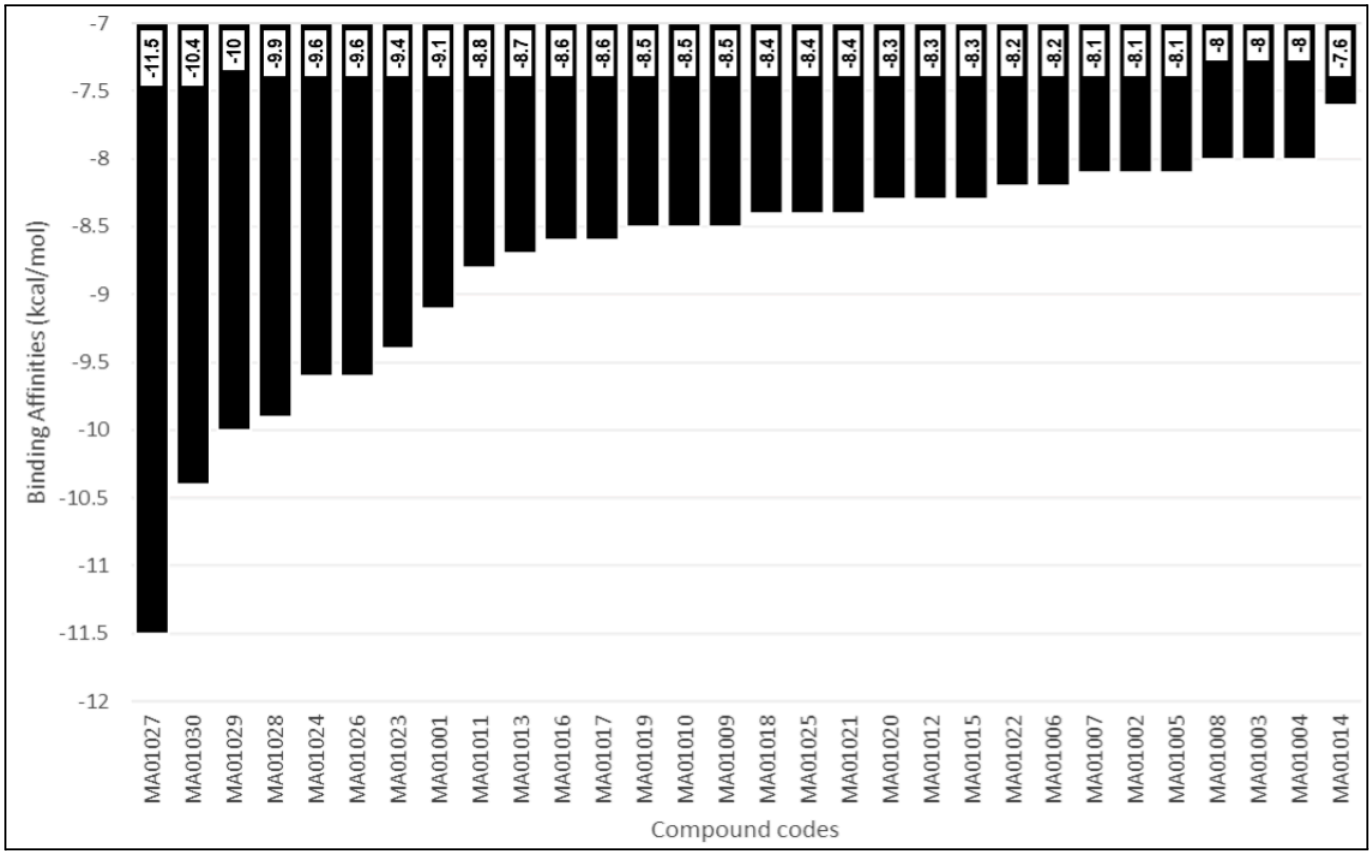

Figure 2. Ranking the compounds based on docking scores. Histogram showing the binding affinity values in kcal/mol. for the 30 compounds that were screened in this study.

Among the 30 compounds chosen from a focused library in the ZINC database (Figure 1), MA01027 was identified as the hit compound based on its highest binding affinity to the CagA receptor using SwissDock. As shown in Figure 2, the binding affinity values of the compounds ranged from -11.5 kcal/mol. (highest binding affinity for MA01027) to -7.6 kcal/mol. (lowest binding affinity for MA01014) with almost 4 kcal/mol. difference. The structures of compounds used in this study are diverse along with some stereoisomers within the focused library. The stereoisomeric sets of compounds include set-1 (MA01002, MA01003, MA01004 and MA01005), set-2 (MA01006, MA01007, MA01008 and MA01009), set-3 (MA01010, MA01011, MA01012 and MA01013) and set-4 (MA01028 and MA01029). The binding affinities for sets-1, 2 and 3 were found to be relatively lower (-8 kcal/mol. to -8.8 kcal/mol.) when compared to the set-4 compounds (-9.9 kcal/mol. to -10 kcal/mol.). The internal variability of binding affinities within the sets of these stereoisomers (-0.1 kcal/mol. to -0.8 kcal/mol.) was not considerable. Besides the stereoisomers, three sets of analogs with different common core moieties were also present within the focused library viz. analog set-1 (MA01015, MA01016, MA01017, MA01018, MA01020, MA01021 and MA01022) containing an alpha-piperidone core moiety, analog set-2 (MA01023 and MA01024) containing an anthraquinone core moiety and analog set-3 (MA01025, MA01026, MA01027 and MA01030) containing a core benzene ring. The remaining three compounds, MA01001, MA01014 and MA01019 with binding affinity values of -9.1 kcal/mol., -7.6 kcal/mol. and -8.5 kcal/mol., respectively, were uniquely different from the above mentioned (Figure 1). Considering the highest binding affinity of MA01027 from the analog set-3 compared to the rest of the compounds, the core benzene ring was concluded to be the most preferred moiety for the charge-neutral and long binding pocket of CagA.

When compared to the binding profile of MA01027, most of the remaining compounds in the focused library used in this study show only partial occupancy of the CagA binding pocket preferentially towards one end rather than the middle of the pocket (Figure 3). Two compounds MA01011 (binding affinity: -8.8 kcal/mol.) and MA01016 (binding affinity: -8.6 kcal/mol.) were bound in the middle of the CagA binding pocket but with lower binding affinities than MA01027 (binding affinity: -11.5 kcal/mol.).

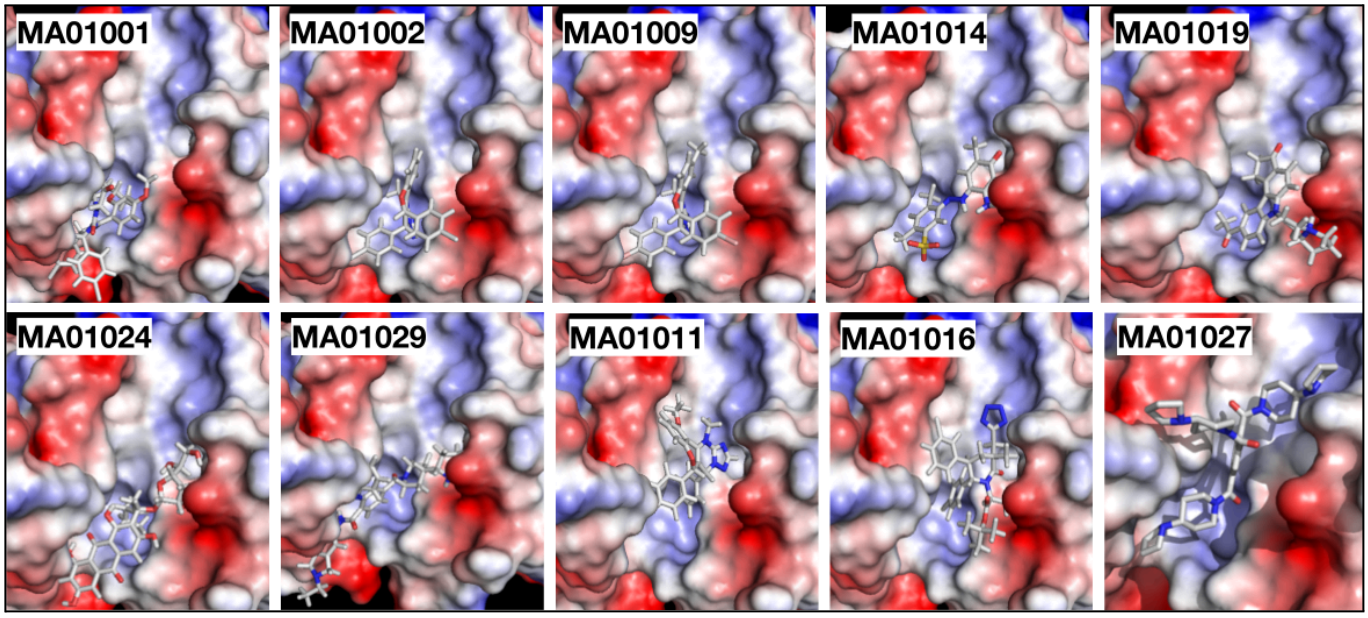

Figure 3. Binding profiles of compounds in CagA binding pocket. All compounds in the top row including MA01024 and MA01029 in the bottom row are bound partially in the long CagA binding pocket suggesting a reason for their lower binding affinity compared to the lead compound, MA01027 shown in the bottom row, rightmost panel. The two compounds, MA01011 and MA01016 in the bottom row are bound in the middle of the CagA binding pocket. However, both MA01011 and MA01016 have lower binding affinities compared to MA01027.

In conclusion, out of the 30 compounds virtually screened in this study, MA01027 was considered as the lead molecule that should be synthesized and evaluated further in future.

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Conflict of interest: The authors declare no conflict of interest in this study.

Author contributions: M.A. performed virtual screening and analyzed the binding profiles of all molecules. R.S.Y. is the principal investigator who supervised M.A., secured required material for the project, provided the laboratory space and facilities needed. R.S.Y. wrote, edited and finalized the manuscript. All authors approve the final manuscript.

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