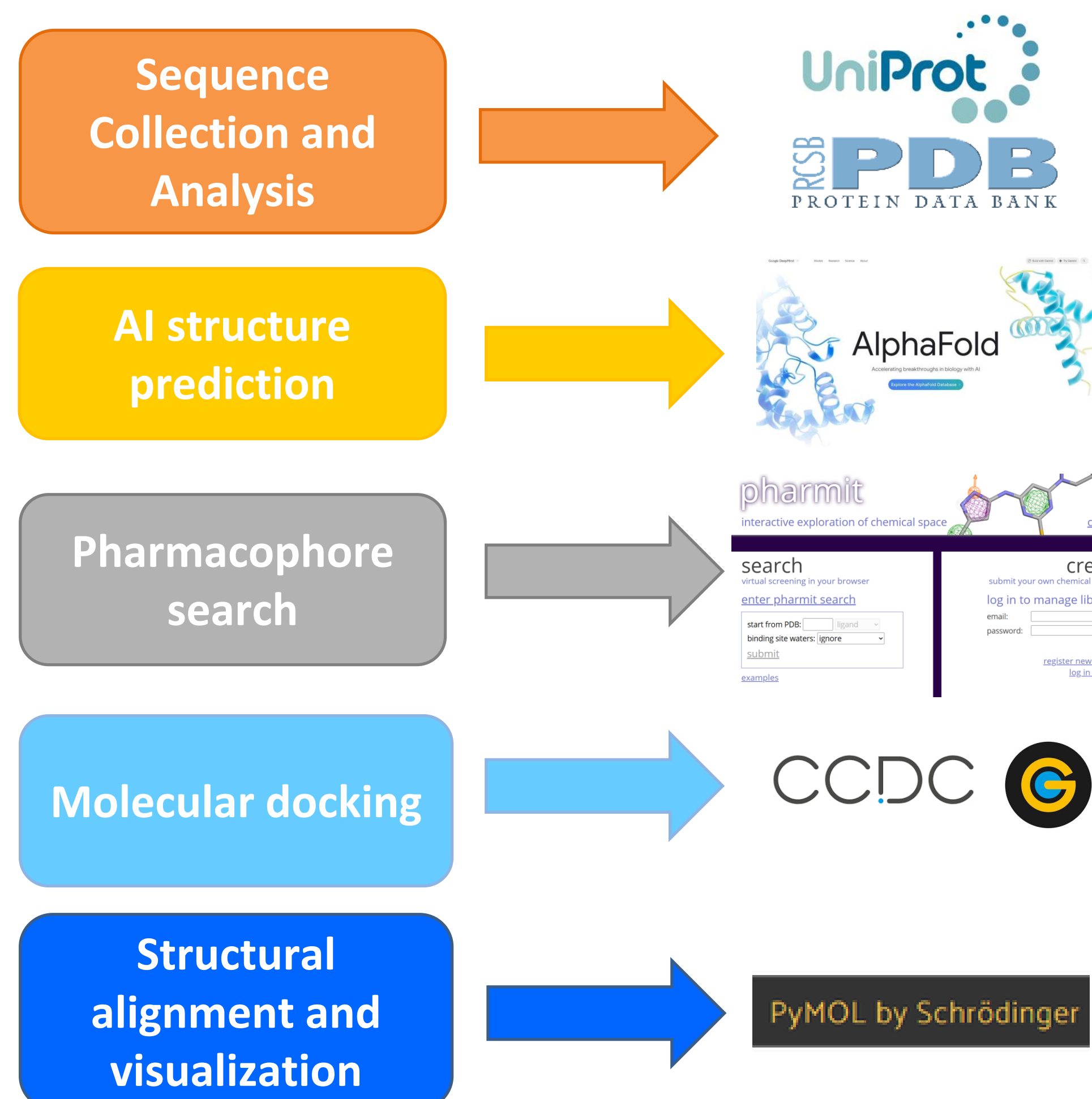


INTRODUCTION

Microtubule-associated serine/threonine kinases (MAST) are linked to a broad spectrum of diseases, including cancer, neurological disorders, inflammatory bowel disease, and type 2 diabetes; however, no selective inhibitors of MAST family members have been identified to date. The MAST family comprises five members — MAST1–4 and MASTL (GWL) — and represents a promising but underexplored therapeutic target. Most kinase inhibitors target the ATP-binding pocket, which is highly conserved throughout the kinome, making selectivity difficult to achieve. Prior work revealed that the ATP-binding site is fully conserved among MAST subfamily members, offering no basis for selective targeting. The PIF pocket, an allosteric site shared by AGC kinases but with markedly greater amino acid variability, presents a more promising alternative. Its effectors have shown strong selectivity across the AGC superfamily, with established inhibition protocols already validated for PDK1, PKC, and S6K. This study focuses on virtual screening for MAST-selective PIF pocket inhibitors, with subsequent analysis of isotype-binding variability across MAST1–4 and MASTL.

METHODS



RESULTS

Primary pharmacophore screening identified 23 hits from virtual screening. All selected compounds showed preferential binding to the PIF pocket among MAST family members, regardless of additional affinity for the ATP or K pockets. Predicted affinity for MASTL was consistently lower than for other family members. Among the Enamine compounds, Z15749680 demonstrated the best overall binding parameters and serves as the primary reference for combinatorial optimization and in vitro testing, while Z279671592 was identified as the most promising candidate for isotype-neutral inhibition. SBF1 showed strong affinity for the PIF and K sites of MAST1/3/4 — peaking at the MAST4 PIF site — but minimal interaction with the MASTL PIF site and the MAST2 ATP pocket. Control compound PS423 displayed the highest affinity for the PIF sites of MAST1–3, while Z87739851 was selective for MAST4, and Z19748016 showed elevated affinity for MAST2 and MAST4. Z318373636 and Z56794203 were most active at the MAST1/3 and MAST1–3 PIF sites, respectively, and Z18477561 showed no predicted interaction with the MAST3 ATP-binding site.

RESULT

No PIF-effectors have been reported for MAST family kinases, despite this pocket being one of the most experimentally validated sites of allosteric inhibition in the AGC family. Initial characterization of the MAST PIF pocket used reference ligand 78W (from PDK1 structure 5LVO), identifying 13 residues within 6 Å across all family members. Sequence variations detected at these positions (K/LI/I/[AV]5F/R/SF[ED]10/LCM) suggest potential isotype-specific differences in ligand binding. As no experimental MAST kinase structures exist, complexes of MAST1–4 and GWL with 78W were reconstructed using the AlphaFold3-based Protenix server. A generalized pharmacophore was derived and used for a Pharmit server virtual screening of the Enamine Ltd. library (60.5 million conformers for 4.1 million compounds). Pharmacophore screening with steric constraints and standard filters (MaxScore=0; Max mRMSD=1; Single conformer=ON) yielded 23 hits. Subsequent AI-assisted complex reconstruction identified three binding sites within the MAST catalytic domain: the allosteric PIF pocket, the ATP-binding site, and the K-pocket (Fig. 2).

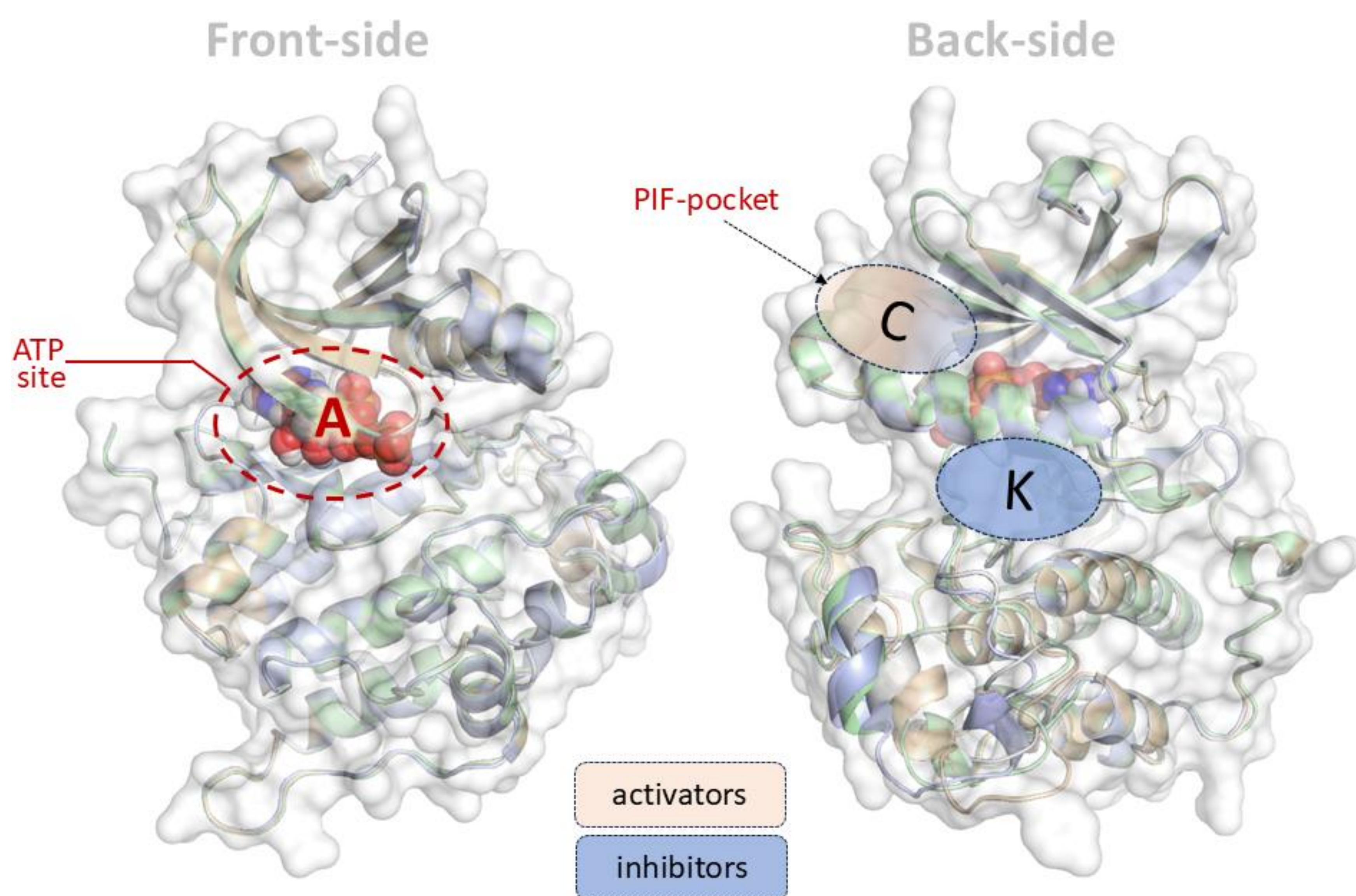


Figure 1. Ligand-binding pockets predicted for pharmacophore search leaders, based on artificial intelligence-driven complex reconstruction (AlphaFold3 + Protenix). **A** — ATP site, **C** — PIF pocket, and **K** — the pocket predicted by homology to the Aurora-A/TPX2 complex (reference PDB structure: 5ORL).

The selected hits were validated by molecular docking in CCDC GOLD (ChemPLP/ASP fitness functions; Genetic Algorithm, Search Efficiency=200%). Crystallographically confirmed AGC kinase inhibitors were used as positive control. Up to 10 hypotheses for each compound were ranked based on ChemPLP score.

	PIF					ATP					K				
	MAST1	MAST2	MAST3	MAST4	MASTL	MAST1	MAST2	MAST3	MAST4	MASTL	MAST1	MAST2	MAST3	MAST4	MASTL
9EJ	-68,81	-76,7	-76,7	-84,87	-65,79	-77,86	-78,37	-80,13	-79,36	-65,91	-64,04	-73,45	-51,69	-79,33	-44,4
SBF1	-54,67	-61,45	-80,02	-93,79	147,71	-75,79	-74,67	-72,37	-75,1	-75,37	-81,3	-78,22	-70	-52,8	-52,8
PS48	-64,18	-67,85	-67,83	-59,69	-48,5	-52,18	-53,91	-54,54	-53,92	-50,51	-55,03	-55,24	-53,98	-53,1	-41,9
PS171	-59,52	-63,82	-59,51	-64,65	-56,87	-57,34	-57,49	-59,09	-59,3	-58,07	-52,14	-56,3	-51,31	-57,07	-42,02
PS432	-69,81	-70,28	-70,28	-68,44	-34,13	-63,4	-62,01	-56,02	-68,24	-59,73	-54,91	-62,35	-48,24	-58,19	-49,63
PS77	-70,02	-71,53	-71,78	-69,71	-54,57	-61,99	-63,33	-59,91	-61,72	-62,04	-70,23	-73,47	-55,37	-59,58	-44,53
PS423	-83,01	-86,38	-86,38	-75,82	-57,88	-61,53	-66,44	-61,55	-61,6	-63,51	-66,59	-71,61	-60,48	-58,58	-45,25
Z18477561	-56,96	-56,69	-63,95	-68,53	-54,68	-60,92	-57,89	28,86	-56,6	-46,64	-65,91	-62,47	-48,49	-62,07	-55,37
Z1982253314	-64,29	-63,82	-64,12	-58,14	-55,24	-60,81	-62,53	-62,95	-61,75	-55,38	-55,74	-52,63	-47,16	-61,69	-40,89
Z749631192	-72,69	-73,15	-73,15	-56,13	-52,25	-60,55	-60,63	-62,43	-60,55	-55,57	-52,07	-52,34	-46,47	-60,5	-42,74
Z240223836	-64,56	-63,77	-64,49	-64,91	-52,74	-59,26	-65,18	-58,74	-58,43	-54,54	-62,06	-58,26	-46,89	-59,21	-49,87
Z4178366809	-60,81	-62,37	-68,49	-66,52	-52,96	-64,08	-66,65	-61,34	-63,48	-53,27	-66,15	-62,56	-50,57	-62,56	-48,84
Z87739851	-46,51	-42,99	-48,23	-57,61	-47,66	-57,34	-59,63	-57,05	-57,05	-48,25	-51,22	-56,48	-47,14	-57,58	-47,83
Z318366728	-65,81	-68,22	-68,22	-56,81	-51,63	-56,99	-57,56	-58,9	-57,85	-51,63	-52,54	-55,39	-55,18	-56,78	-39,2
Z56881118	-56,06	-58,76	-58,76	-63,2	-49	-56,84	-55,19	-52,61	-53,11	-50,19	-62,51	-59,37	-46,89	-51,82	-42,53
Z784943602	-59,74	-62,31	-65,77	-59,73	-54,41	-56,81	-60,05	-58,88	-55,24	-53,86	-57,32	-58,05	-50,46	-56,28	-40,54
Z344153008	-68,94	-70,17	-70,6	-57,93	-53,27	-56,19	-50,22	-53,57	-57,3	-53,13	-50,32	-54,53	-48,49	-57,64	-44,71
Z19748016	-50,18	-55,2	-63,37	-58,27	-46,76	-56,05	-60,5	-54,06	-56,9	-49,37	-59,19	-56,94	-47,18	-56,66	-43,22
Z87740098	-50,19	-48,4	-50,17	-57,45	-47,29	-55,93	-59,12	-48,83	-56,75	-48,39	-59,69	-57,5	-52,29	-56,21	-38,6
Z15749680	-76,66	-79,13	-79,13	-68,9	-62,63	-72,7	-73,4	-63,59	-72,15	-65,5	-65,77	-77,11	-53,77	-65,68	-54
Z318373636	-63,42	-59,33	-63,63	-53,98	-47,4	-51,86	-53,03	-53,44	-52,97	-47,57	-50,36	-52,11	-56,28	-53,42	-38,98
Z87739734	-54,53	-53,96	-54,49	-55,54	-46,27	-51,42	-57,42	-50,75	-51,21	-48,52	-52,09	-54,75	-51,19	-51,42	-41,58
Z1885603236	-52,58	-65,75	-65,75	-60,17	-55,79	-51,03	-50,2	-52,58	-52,81	-52,39	-55,75	-55,55	-36,61	-52,83	-38,2

Figure 2. Heatmap of ChemPLP scores for selected compounds, calculated based on docking into the PIF, ATP, and K sites. Docking and fitness score ranking were performed using CCDC GOLD.

Note: NO — no binding pose predicted.

CONCLUSIONS

It was concluded that PIF pocket represents a viable site for selective inhibition of MAST family members, and it was confirmed by isotype-specific differences in ligand binding profiles. Virtual screening of the Enamine Ltd. library combined with AI-assisted structural reconstruction and CCDC GOLD docking identified Z15749680 as the lead compound, and Z279671592 as the most promising isotype-neutral candidate. The consistently lower predicted affinity for MASTL across all compounds suggests structural features of the MASTL PIF pocket that distinguish it from other family members, and it may be base for future isoform-selective design. These findings establish a foundation for experimental validation and structure-based optimization of MAST-selective PIF pocket inhibitors.

ACKNOWLEDGEMENTS

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