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# PREDICTION OF PROTEIN-LIGAND BINDING SITES MODULATING ACTIVITY OF MAST PROTEIN KINASES

***Aim.*** Identification of the protein-ligand binding sites, that may be the target of compounds, affecting individual human protein kinases of the MAST family (MAST1, 2, 3, 4 and MASTL / GWL). ***Methods.*** Literature and database search. Comparison of protein and ligand structures. Protein structure modeling, structural superimposition, etc. ***Results***. The structural alignment demonstrates significant similarity of catalytic domains in MAST1, 2, 3, 4 and MASTL (GWL). It justifies transferring of reference ligands from PDB structures to human MASTs, discovering potential sites of ligand binding. 13 sites of ligand-binding were specified based on refrence ligands, transferred from RCSB Protein Data Bank structures, and differences in sites amino acid composition of MAST family members were discovered. ***Conclusions.*** Based on the differences in the amino acid composition of the studied pockets in MAST1, 2, 3, 4 and MASTL (GWL), the sites B, C, D, E, F, were selected for further study and virtual screening for new selective inhibitors of individual members of MAST protein kinase family.

*Keywords*: MAST family, protein kinases, inhibitors, ligands, binding site.

**M**icrotubule-**A**ssociated **S**erine/**T**hreonine (MAST) kinases represent an evolutionary conserved branch of the AGC protein kinase superfamily [1, 2]. Being involved in fundamental processes in the cell, MAST kinases are also implicated in a wide range of diseases, including cancer, inflammatory diseases and neurological disorders [1]. In general, the mechanisms explaining MAST kinases involvement in human diseases remain rather obscure. Inhibitors are important instruments helping us to understand functions of protein kinases, as well as the perspective base for diseases treatment. Depending on binding site, kinase inhibitors are classified as types I-IV [3]. Type I (the majority of compounds) bind to the ATP-binding pocket, type II occupies the ATP site and an allosteric pocket, that opens when the kinase is inactive, type III binds adjacent to ATP pocket, type IV bind N or C kinase lobes or other domains. However, the almost identical ATP-binding site of all AGC kinases is the cause of non-specificity of Type I inhibitors, making quest for alternative sites and compounds an extremely important task.

The MAST family is divided into two subfamilies: MAST and MASTL. MAST subfamily includes 4 kinases (MAST1-4). MAST1 and MAST2 appears to link the dystrophin/utrophin network to microtubular filaments via the syntrophin proteins [4]. Additionally, MAST2 involved in spermatid maturation, regulates lipopolysaccharide-induced IL-12 synthesis in macrophages [5]. MAST3 involved in regulating the immune response, cytoskeletal organization, signal transduction, and peptidyl-serine phosphorylation [6]. MAST4 regulates self-renewal of spermatogonial stem cells [7], embryonic brain development and neurodevelopmental disorders [8]. Subfamily MASTL consists of one kinase, acting as a regulator of mitosis entry and maintenance. MASTL indirectly inhibits PP2A mediating phosphorylation and activation of ARPP19 and ENSA. Also, it may be involved in megakaryocyte differentiation [9].

The objective of the study was identification of the protein-ligand binding sites, that may affect human protein kinases of MAST family (MAST1, 2, 3, 4 and MASTL/GWL). It comprised several points: 1) structural comparison of kinase domains and sites, 2) transfer of reference ligands from PDB structures to the models of human MAST kinases for prediction of potential pockets of sites and their a.a. composition, 3) study of a.a. variations in selected sites of different family members. The purpose of last one, was to provide us information on

potential variations in sites of individual MAST family members.

**Materials and methods**

The amino acid sequences of human MAST kinases were obtained from the UniProtKB (www.uniport.org): MAST1_HUMAN (Q9Y2H9), MAST2_HUMAN (Q6P0Q8), MAST3_HUMAN (O60307), MAST4_HUMAN (O15021) and MASTL=GWL_HUMAN (Q96GX5) [5, 10]. Sequences alignments were possessed in ClustalX v. 2.1 (www.clustal.org).

RCSB Protein Data Bank (www.rcsb.org) was used as a source of structural information. The list of PDB-complexes used as a reference, were collected based on the literature: 4M15 (QWS), 5LVO (78W), 3ROC (29B), 3PYY (3YY), 6FVF (503), 3JVR (AGX), 5OTZ (AUT), 6EHK (AUW), 5OSJ (AAK), 6BKW (FPZ), 5ORL (A4W) and 6D1Z (FQD). AlphaFold [11] structures used in research: MAST1 – AF-Q9Y2H9-F1; MAST2 – AF-Q6P0Q8-F1; MAST3 – AF-O60307-F1; MAST4 – AF-O15021-F1 and MASTL – AF-Q96GX5-F1. PyMOL V.2.5.4 (Schrödinger, LLC – www.pymol.org) was used for structural analysis and visualization. Similarity of structures were estimated based on RMSD score of Cα atoms. Amino acids (a. a.) of sites pockets were specified based on 4-5 Å distance from reference ligands transferred from PDB-structures.

**Results and discussion**

The most of kinase inhibitors interact with ATP-binding site (type I inhibitors), conserved across diverse kinase families At the same time, it is no selective type I inhibitors of MAST family members [12]. It provokes our interest in more selective effectors of types II and III. The compounds of Type II, bind to the ATP pocket, but also expands interactions to adjacent hydrophobic areas [13]. The inhibitors of Type III interact with distant ahydrophobic pockets, inducing conformational shifts suppressing catalytical activity. The initial point for our study, grounded on the revision of known sites of ligand-dependent regulation of protein kinases [3]. The reference PDB structures, used in [3], were also used in current research, however, some points were clarified in prosses (Table).

The structural alignment of MAST1, 2, 3, 4 and MASTL (GWL) displays considerable similarity of their catalytic domains (RMSD=0-0242). It justifies transferring of reference ligands from PDB structures to human MASTs discovering topology of probable sites (Table, Fig. 1). With this approach, we studied 13 known and predicted sites of ligand-binding in MAST-kinases and compared their a.a. composition among family members.

**Pocket A** (Fig. 1 A, 2) – the site of ATP-cleft (ATP-binding site). Its a.a. composition revealed identity in all MASTs, making selective inhibition impossible. It was consistent with the literature data, claiming similar inhibition effect on all AGC protein kinases and some related families (Aurora, PLK, etc.).

Table. RCSB Protein Data Bank structures used for prediction of potential ligand-binding sites

| **Pocket** | **Inhibitor /activator type** | **Reference structures from RCSB Protein Data Bank** | | |
|---|---|---|---|---|
| | | **kinase** | **PDB ID** | **ligand ID** |
| **B**+A | type III, VI | ITK | 4M15 | QWS |
| C | type IV, VI, activators | PDPK1 | 5LVO | 78W |
| D | type IV, VI, activators | MAPK14 | 3ROC | 29B |
| E | type IV, activators | ABL1 | 3PYY | 3YY |
| F | type IV, activators | CSNK2A1 | 6FVF | 503 |
| G | type IV | CHK1 | 3JVR | AGX |
| H | type IV | CSNK2A1 | 5OTZ | AUT |
| **H**+A | type V | CSNK2A1 | 6EHK | AUW |
| I | type VI | CDK2 | 5OSJ | AAK |
| J | type IV | BTK | 6BKW | FPZ |
| K | type IV, VI | AURKA | 5ORL | A4W |
| L | type IV | NTRK1 | 6D1Z | FQD |

*Notes*: A – is the site of ATP-cleft – ATP-binding site, interacting with inhibitors of type I.

**Pocket B** (Fig. 1 A, 2) was predicted based on reference QWS (4-(carbamoylamino)-1-[7-(propan-2-yloxy)naphthalen-1-yl]-1H-pyrazole-3-carboxamide) transferred from PDB structure 4M15 of ITK kinase (interleukin-2-inducible T-cell kinase) [14]. QWS dually bound to allosteric pocket and the ATP site [14]. The a.a. environment of reference ligand with a distance of 4 Å revealed 19 a.a. for all MAST kinases: [AV][1]/F/V/E/R/D/I/L/T/F/A/V/[SD][13]/M/S/L/T/D/F. The variations were noticed only in 1st and 13th a.a positions. In MAST2, 4 and MASTL, this site demonstrate identity. In MAST1 in 13th position, polar and uncharged Ser (S) was substituted with negatively charged Asp (D). The equivalent substitution of hydrophobic Ala / Val in the 1st position of MAST3 is insignificant. In view of that, some differ in ligand binding can be expected only in MAST1.

**Pocket C (**PIF-Pocket, see Fig. 1 B, 2) was predicted based on reference ligand 78W = PSE10 (2-oxidanylidenepropyl ~{N}-(2-chloranyl-6-fluoranyl-phenyl)carbonyl-~{N}'-(4-chlorophenyl) carbamimidothioate) transferred from structure 5LVO of human PDK1 (PDPK1) kinase. The a.a. environment of 78W with a distance of 5 Å revealed 13 a.a. for all MAST kinases: K/LI/I/[AV][5]F/R/SF[ED][10]/LCM. The variations were identified only in 5th and 10th a.a. positions of the Pocket C. In MAST1, 4 and MASTL, the site was identical. In 10th position of MAST2, negatively charged Glu (E / -4.71) is substituted with slightly less negatively charged Asp (D / -3.71). Substitution of hydrophobic Ala-5 on Val of MAST3 is insignificant. Thus, specific PIF-Pocket effectors of individual MAST kinases are unsure. Some variance in ligand binding can be expect only in the case of MAST2 (Fig. 2.).

**Pocket D** (Fig. 1 A, 2) was predicted based on reference ligand 29B (4-[4-(4-fluorophenyl)-1H-pyrazol-3-yl]pyridine) transferred from PDB structure 3ROC of MAPK14 kinase. The a.a. environment of reference ligand with a distance of 5 Å revealed 11 a.a. for all MAST kinases – R/PE/[LF][4]RQ/[IV][7]/[QT][8][NS][9]P/R, and 4 a.a. variations. In MAST2, 4 and MASTL, Pocket D was conserved. In MAST1, polar and uncharged Gln (Q) in position "8" is substituted by similar, but shorter Thr (T). It may affect site volume, but is unlikely to be critical for interaction with ligands. Much more differences were in MAST3, demonstrating 3 substitutions at once: 1) hydrophobic Leu in 4th position is substituted by hydrophobic, but longer and aromatic Phe (F), 2) polar and uncharged Asn (N) in position "9" is substituted by similar, but shorter Ser (S), and 3) minor substitution of hydrophobic Val-7 (V) on Ala (A). The first two substitutions, in our view, are significant, since the larger volume and unique cyclic fragment, in the case of MAST3, opens up the opportunities of specific interactions.

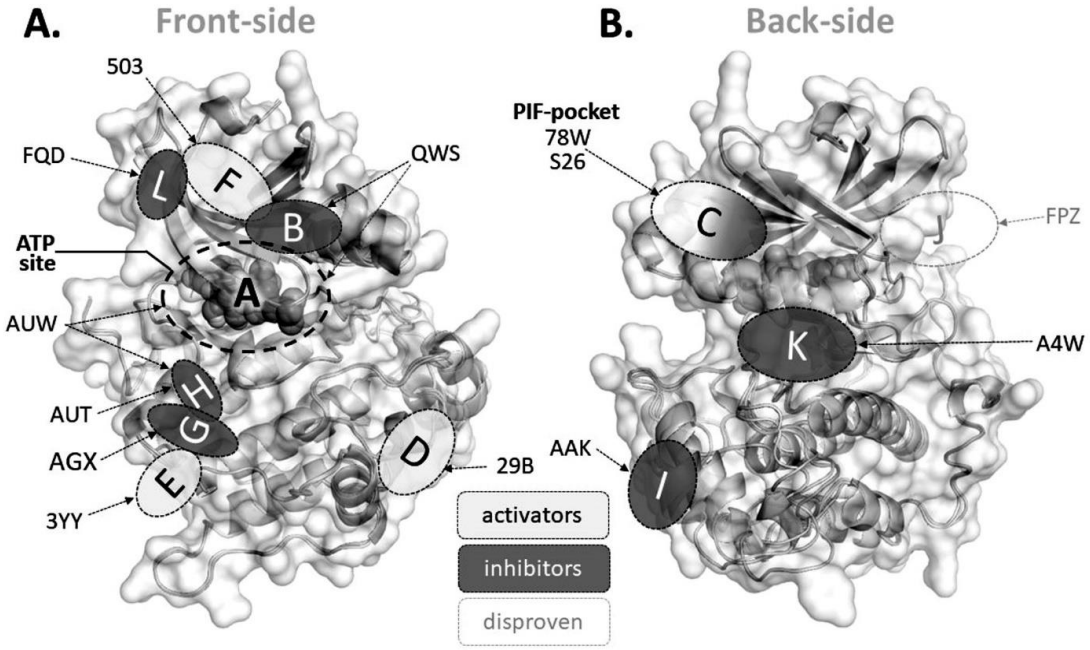


Fig. 1. Ligands-binding pockets: front-side view focusing on the ATP site (red) and a back-side view. For each pocket (A, B, C, D, E, F, G, H, A+H, K, L, I), the PDB-numbers of reference ligands are specified [3].

**ATP-SITE, 4 Å (ATP and STU)**

```
MAST1: ISNGAYGAV-A-K---MEYV--GD-DN-L-TD
MAST2: ISNGAYGAV-A-K---MEYV--GD-DN-L-TD
MAST3: ISNGAYGAV-A-K---MEYV--GD-DN-L-TD
MAST4: ISNGAYGAV-A-K---MEYV--GD-DN-L-TD
MASTL: ISNGAYGAV-A-K-E-LSYAK-GE-EN-L-TD
```

**Pocket B 4 Å (QWS: 4-(carbamoylamino)-1-[7-(propan-2-yloxy)naphthalen-1-yl]-1H-pyrazole-3-carboxamide)**

```
MAST1: AFVERDILTFA-VDM-S-LTDF
MAST2: AFVERDILTFA-VSM-S-LTDF
MAST3: VFVERDILTFA-VSM-S-LTDF
MAST4: AFVERDILTFA-VSM-S-LTDF
MASTL: AFVERDILTFA-VSM-S-LTDF
```

**Pocket C / PIF-pocket 5 Å (78W: 2-oxidanylidenepropyl ~{N}-(2-chloranyl-6-fluoranyl-phenyl)carbonyl-~{N}'-(4-chlorophenyl)carbamimidothioate)**

```
MAST1: K-LI-I-AF-R-SFE-LCM
MAST2: K-LI-I-AF-R-SFD-LCM
MAST3: K-LI-I-VF-R-SFE-LCM
MAST4: K-LI-I-AF-R-SFE-LCM
MASTL: K-LI-I-AF-R-SFE-LCM
```

**Pocket D 5 Å (29B: 4-[4-(4-fluorophenyl)-1H-pyrazol-3-yl]pyridine)**

```
MAST1: R-PE-LRQ-I-TNP-R
MAST2: R-PE-LRQ-I-QNP-R
MAST3: R-PE-FRQ-V-QSP-R
MAST4: R-PE-LRQ-I-QNP-R
MASTL: R-PE-LRQ-I-QNP-R
```

**Pocket E 5 Å (3YY: (5R)-5-[3-(4-fluorophenyl)-1-phenyl-1H-pyrazol-4-yl]imidazolidine-2,4-dione)**

```
MAST1: ALPVEMARM-FA-L-FLV-LP-F
MAST2: ALPVDMVRL-FA-L-FLV-LP-F
MAST3: PLPVDMARL-FA-L-FLV-LP-F
MAST4: PLPVDMARM-FA-L-FLV-PP-F
MASTL: PLPVDMARM-FA-L-FLV-PP-F
```

**Pocket F 5 Å (503: [1-[2-(phenylsulfonylamino)ethyl] piperidin-4-yl]methyl 5-fluoranyl-2-methoxy-1~{H}-indole-3-carboxylate)**

```
MAST1: CENDF-T-YGA-Y-R-M-KINK-N-F-T-RHLC
MAST2: SEEDF-T-YGA-F-K-M-KINK-N-F-T-RHLC
MAST3: CESDF-T-YGA-Y-R-I-KINK-N-F-T-RHLC
MAST4: RESDF-T-YGA-Y-K-M-KINK-N-F-T-RHLC
MASTL: RESDF-T-YGA-Y-K-M-KINK-N-F-T-RHLC
```

**Pocket G 5 Å (AGX: (1S)-1-(1H-benzimidazol-2-yl)ethyl (3,4-dichlorophenyl)carbamate)**

```
MAST1: ATLLKNIG-P-Y-E-GCV
MAST2: ATLLKNIG-P-Y-E-GCV
MAST3: ATLLKNMG-P-Y-E-GCV
MAST4: ATLLKNMG-P-Y-E-GCV
MASTL: ATLLKNMG-P-Y-E-GCV
```

**Pocket H 5 Å (AUT: [3,5-bis(chloranyl)-4-(2-ethylphenyl) phenyl]methanamine)**

```
MAST1: GDCATLL-L-A-YF-T-KPDNLLI-IL-EF
MAST2: GDCATLL-L-V-YF-T-KPDNLLI-IL-EF
MAST3: GDCATLL-L-A-YF-T-KPDNLLI-VL-EF
MAST4: GDCATLM-L-A-YF-T-KPDNLLV-IL-EF
MASTL: GDCATLM-L-A-YF-T-KPDNLLV-IL-EF
```

**Pocket H+A 5 Å (AUW: 2-(1~{H}-benzimidazol-2-yl)-~{N}-[[3,5-bis(chloranyl)-4-(2-ethylphenyl)phenyl]methyl] ethanamine)**

```
MAST1: IS-V-GDCATLL-L-A-YF-T-KPDNLLI-T-IL-EF
MAST2: IS-V-GDCATLL-L-V-YF-T-KPDNLLI-T-IL-EF
MAST3: IS-V-GDCATLL-L-A-YF-T-KPDNLLI-T-VL-EF
MAST4: IS-V-GDCATLM-L-A-YF-T-KPDNLLV-T-IL-EF
MASTL: IS-V-GDCATLL-L-A-YF-T-KPDNLLV-T-IL-EF
```

**Pocket I 5 Å (AAK: ~{tert}-butyl 4-propanoyl-2,3-dihydroquinoxaline-1-carboxylate)**

```
MAST1: IE-AR-FL-E-RQGY-PL
MAST2: IE-AR-FL-E-RQGY-PL
MAST3: IE-AR-FI-E-RQGY-PL
MAST4: IE-AR-FL-E-RQGY-PL
MASTL: IE-AR-FL-E-RQGY-PL
```

**Pocket J 5 Å (FPZ: triphenylphosphane)**

```
MAST1: IE-AR-FL-E-RQGY-PL
MAST2: IE-AR-FL-E-RQGY-PL
MAST3: IE-AR-FI-E-RQGY-PL
MAST4: IE-AR-FL-E-RQGY-PL
MASTL: IE-AR-FL-E-RQGY-PL
```

**Pocket K 5 Å (A4W: ~{N}-(3-chloranyl-2-fluoranyl-phenyl)-3-sulfanyl-propanamide)**

```
MAST1: DI-FA-YLH-YGI-M
MAST2: DI-FA-YLH-YGI-I
MAST3: DI-FA-YLH-YGI-I
MAST4: DI-FA-YLH-YGI-V
MASTL: DI-FA-YLH-YGI-V
```

**Pocket L 5 Å (FQD: 5-(4-fluorophenyl)thieno[2,3-d]pyrimidin-4(3H)-one)**

```
MAST1: Y-K-I-Q-QA-ER-IL-A-VVC-M-M-VHRD-LTDFGLS
MAST2: Y-K-I-Q-QA-ER-IL-A-VVS-M-M-VHRD-LTDFGLS
MAST3: Y-K-I-Q-QV-ER-IL-A-VVS-M-M-VHRD-LTDFGLS
MAST4: Y-K-I-Q-QA-ER-IL-A-VVS-M-M-VHRD-LTDFGLS
MASTL: Y-K-I-Q-QA-ER-IL-A-VVS-M-M-VHRD-LTDFGLS
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Fig. 2. Analysis of amino acid composition of known and predicted sites of ligand binding in human protein kinases MAST1, MAST2, MAST3, MAST4 and *MASTL (GWL)*. Amino acids were sampled based on 5Å distance from reference ligands, transferred from PDB structures mentioned in the table.

**Pocket E** (Fig. 1 A, 2) was predicted based on reference 3YY ((5R)-5-[3-(4-fluorophenyl)-1-phenyl-1H-pyrazol-4-yl]imidazolidine-2,4-dione) transferred from PDB structure 3PYY of ABL1 kinase. The a.a. environment of reference ligand with a distance of 5 Å revealed 18 a.a. for all MAST kinases: [PA][1]LPV[DE][5]M[AV][7]R[ML][9]/-FA/L/FLV/PP/F. In MAST4 and MASTL site was identical. MAST3 bears minor substitution Leu/Met in 9$^{th}$ position, also identified in MAST2. Last one is the most differ from other MAST kinases thanks to substitutions Pro/Ala in 1$^{st}$ and Ala/Val in 7$^{th}$

positions. Substitution of cyclic Pro by Ala, also found in MAST1, seems the most essential. At the same time, substitution at 5th position of negative Glu/Asp in MAST1, is not critical for ligand binding. In general, for Pocket E, we expect differences in ligand binding between the two groups: I) MAST1/2, and II) MAST3/4/L.

**Pocket F** (Fig. 1 A, 2) was predicted based on reference ligand 503 = CCh503 ([1-[2-(phenylsulfonylamino)ethyl]piperidin-4-yl]methyl-5-fluoranyl-2-methoxy-1~{H}-indole-3-carboxylate)) transferred from PDB structure 6FVF of CK2α, and initially, it was identified as an allosteric inhibitor disrupting the CK2α/CK2β interfaces of the CK2 heterotetramer [15]. Consequently, the role of this site in MAST kinases requires clarification. However, a homologous pocket was detected, and 5 Å environment from reference ligand revealed 23 a.a. for all MAST kinases: [RGSC][1]E[SNE][3]DF/T/YGA/[YF][10]/[KR][11]/[MI][12]/KINK/N/F/T/RHLC. In MAST4 and MASTL, this site was identical. Variations in the rest – MAST1, 2 and 3, are quite significant and can induce variations in the local charges, volumes and hydrophobic/hydrophilic properties of the Pocket F, identifying it as important for further study.

**Pocket G** (Fig. 1 A, 2) was predicted based on reference ligand AGX ((1S)-1-(1H-benzimidazol-2-yl)ethyl (3,4-dichlorophenyl)carbamate) transferred from allosteric site of Checkpoint kinase 1 (CHK1, PDB: 3JVR). Initially, this interaction is reversible and noncompetitive. The environment (5 Å) of reference ligand revealed 14 a.a. for all MAST kinases: ATLLKN[MI][7]G/P/Y/E/GCV. The sole variation is a similar Met/Ile substitution at the 7th position. Consequently, variations in binding with MASTs Pocket G is doubtful.

**Pocket H** (Fig. 1 A, 2) was predicted based on reference ligand AUT = CAM4712 ([3,5-bis(chloranyl)-4-(2-ethylphenyl)phenyl]methanamine) transferred from PDB structure 5OTZ of CK2α subunit of Casein kinase II. The 5 Å environment of reference ligand revealed 23 a.a. for all MAST kinases: GDCATL[LM][7]/L/[AV][9]/YF/T/KPDNLLI/[IV][20]L/EF, and confirmation of its existence is required. The similarity of the kinase domains suggests that it is possible. At the same time, we predict identical effects of ligand binding for all MAST kinases. It's reasoned by the fact, that noticed variations (Leu/Met, Ala/Val and Ile/Val) are extremely close, and, most likely, have no impact on the properties of Pocket H.

**Pocket H+A** (Fig. 1 A, 2) was predicted based on reference ligand AUW (2-(1~{H}-benzimidazol-2-yl)-~{N}-[[3,5-bis(chloranyl)-4-(2-ethylphenyl)phenyl]methyl]ethanamine)) transferred from PDB structure 6EHK of CSNK2A1 kinase (CK2α). The 5 Å environment of reference ligand revealed 27 a.a. for all MAST kinases: IS/V/GDCATL[LM][10]/L/[AV][12]/YF/T/KPDNLLV/T/[IV][24]L/EF. All substitutions were single and comparable in properties: Ala-12 to Val of MAST2, Ile-24 to Val position of MAST3 and Leu-10 to Met of MAST4. Thus, we assume that in the case of Pocket H+A, variations in ligand binding in MAST kinases is impossible.

**Pocket I** (Fig. 1 B, 2) was predicted based on reference ligand AAK (~{tert}-butyl 4-propanoyl-2,3-dihydroquinoxaline-1-carboxylate) – allosteric inhibitor of Cdk2, transferred from PDB structure 5OSJ. The 5 Å environment of reference ligand revealed 13 a.a. for all MAST kinases: IE/AR/F[LI][6]/E/RQGY/PL. In MAST1, 2, 4 and MASTL, Pocket I demonstrate identity. Only in MAST3 in 6th position changed Leu on Ile. Taking into account the almost identical properties of Leu and Ile, it can be reasonably stated that there are no variances in binding properties of Pocket I among human MAST kinases.

**Pocket J** (Fig. 1 B, 2) was selected based on data of Laufkötter et al. (2022) as the site of FPZ (triphenylphosphine) [3]. It should be noted that FPZ, presented as inhibitor. In fact, according to the annotation of original PDB-structure 6BKW, this ligand is part of the technical process. Originally structure 6BKW demonstrate interaction with the ATP-competitive inhibitor DXM. Also, the analysis of interactions of FPZ with Pocket J raises doubts about its potential as a target site for ligand-induced action. We consider Pocket J as incorrect and excluded it from further research.

**Pocket K** (Fig. 1 B, 2). Initially it was predicted as potential site of ligand-based impact on Aurora-A/TPX2 interaction – "Druggable hot-spot fragment screening, and experimentally confirmed site" (see PDB: 5ORL). It should be noted that it is currently unknown whether this interface in MAST kinases is involved in similar protein-protein interactions. Also, in MAST family members, this region is quite highly conserved, making specific binding of ligands rather doubtful.

**Pocket L** (Fig. 1 A, 2) was predicted based on reference ligand FQD (5-(4-fluorophenyl)thieno[2,3-d]pyrimidin-4(3H)-one) from PDB structure 6D1Z of Tropomyosin receptor kinase NTRK1. The 5 Å environment of reference

ligand revealed 27 a.a. for all MAST kinases: Y/K/I/Q/Q[AV]$^{6}$/ER/IL/A/VV[SG]$^{14}$/M/M/VHRD/LTDFGLS. The variations were detected only in 5$^{th}$ and 14$^{th}$ a.a. positions of Pocket L. In MAST2, 4 and MASTL, this site demonstrates identical a.a. In 14$^{th}$ position of MAST1, the polar and uncharged Ser (S) is substituted with aliphatic Gly (G) – the simplest a.a. with a single hydrogen atom as its side chain. Both Ser and Gly are small in size, and canonical charges of their -$NH_3$ groups are 9.05 and 9.58, respectively. Thus, we do not believe that serine/glycine variation will impact ligand binding. Also, the substitution of hydrophobic Ala-5 with similar Val of MAST3 was considered as equivalent. Consequently, we do not expect variations in ligand binding with Pocket L of different protein kinases MAST.

## Conclusions

It was studied pockets of 13 known and predicted sites of ligand-binding in MAST-kinases and compared their amino acid composition among family members. Based on the differences in the amino acid composition of the studied pockets in MAST1, 2, 3, 4 and MASTL (GWL), the pockets of the sites B, C, D, E, F (Fig. 1, 2) were selected as the items of further research and virtual screening for selective inhibitors, acting on individual protein kinases of MAST family.

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## ПРОГНОЗУВАННЯ САЙТІВ ЛІГАНД-ЗАЛЕЖНОЇ МОДУЛЯЦІЇ АКТИВНОСТІ ПРОТЕЇНКІНАЗ РОДИНИ MAST

***Мета.*** Ідентифікація сайтів зв'язування лігандів, що можуть забезпечити індивідуальний вплив на окремих представників протеїнкіназ родини MAST (MAST1, 2, 3, 4 і MASTL / GWL). ***Методи.*** Аналіз літературних джерел та баз даних. Порівняяльний аналіз структур білків, лігандів та їх комплексів. ***Результати.*** Структурне вирівнювання довело значну подібність каталітичних доменів протеїнкіназ MAST1, 2, 3, 4 і MASTL (GWL) людини. Це дозволило визначити перспективні сайти зв’язування лігандів на поверхні протеїнкіназ MAST людини за допомогою референсних лігандів, що були перенесені із PDB-структур. Досліджено кишені 13 відомих і спрогнозованих сайтів зв’язування речовин, визначених за допомогою лігандів, перенесених зі структур RCSB Protein Data Bank. Досліджено варіації амінокислотного складу зазначених сайтів у протеїнкіназ родини MAST. ***Висновки.*** Визначені відмінності в амінокислотному складі досліджених кишень протеїнкіназ MAST1, 2, 3, 4 та MASTL (GWL) людини дозволяють позначити сайти B, C, D, E, F як перспективні для подальшого дослідження з метою визначення інгібіторів, що будуть селективні на рівні окремих протеїнкіназ родини MAST.
*Ключові слова*: родина MAST, протеїнкінази, інгібітори, ліганди, сайти зв’язування.