FLEXIBLE LIGAND DOCKING STUDIES OF MATRIX METALLOPROTEINASE INHIBITORS USING LAMARCKIAN GENETIC ALGORITHM

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ABSTRACT

As important therapeutic drug targets, matrix metalloproteinases (MMPs) have recently attracted great interest in the search for potent and selective inhibitors using computer-aided molecular modelling and docking techniques. Availability of more than 60 X-ray crystal structures or NMR solution structures related to MMPs in Protein Data Bank (PDB) of which more than half of them are in complex with various MMP inhibitors (MMPIs), provides a great opportunity for docking studies. In this study AutoDock 3.0.5 along with its LGA algorithm were used for automated flexible ligand docking of 32 MMPI-MMP complexes and docking accuracy and reliability of the estimated inhibition constants were evaluated. Twenty-six out of 32 docks had RMSD less than 3.0 Å which is considered as well-docked, however, for the most of the cases (15 out of 27), predicted pKi values were considerably overestimated in comparison to experimental values. To improve pKi prediction regarding MMPI-MMP complexes, inclusion of at least one such a complex in calibration of empirical free energy function in the next release of AutoDock is highly recommended.

Keywords: MMPs, MMPIs, Rational drug design, Computer-aided molecular modelling, Flexible ligand docking, AutoDock.

INTRODUCTION

The human matrix metalloproteinases (MMPs) comprise a family of secreted and transmembrane zinc-dependent endopeptidases that degrade the macromolecular components of the extracellular matrices (ECM) and basement membrane components (1). Under normal physiological conditions, the proteolytic activities of enzymes are controlled by tissue inhibitors of matrix metalloproteinases (TIMPs) (2). In pathological conditions, this balance is shifted towards overactivation of MMPs leading to excessive degradation of the matrix components. Excessive MMP activity has been implicated in numerous disease states involving matrix degradation, which include arthritis (3), periodontal diseases (4), osteogenesis imperfecta, Alzheimer's disease (5) and tumor invasion and metastasis (6).

Currently, at least 22 structurally related zinc-dependent members with a broad spectrum of proteolytic activity against several components of the ECM have been identified. Based on their substrate specificities and primary sequence similarities, MMPs have been broadly categorized into five subfamilies which are summarized in table 1 (7). Three-dimensional (3D) structures of many of these MMPs (MMP-1 to MMP-3, MMP-7 to MMP-9 and MMP-11 to MMP-13) have been determined by the X-ray crystallography and NMR techniques. There are more than sixty 3D-structure entries related to MMPs in Protein Data Bank (PDB) (8) of which more than half of them are in complex with various small molecular inhibitors. MMP inhibitors (MMPIs) are consisted of a zinc binding group (ZBG), including but not limited to hydroxamates (the most potent), carboxylates, phosphinates, and thiolates, coupled to a framework with varying numbers of pocket-occupying side chains (9). Availability of this relatively high number of 3D structures in complex with different inhibitors provides a great opportunity for docking studies.

Automated flexible ligand docking is an emerging technology for rational lead discovery based on the receptor structure which is considered rigid (10). The challenge of docking a flexible ligand into a rigid receptor has been taken up by a number of groups; one particularly good outcome is the AutoDock with its Lamarckian Genetic Algorithm (LGA) (11). In the first release of AutoDock, a rapid grid-based method for energy evaluation in conjunction with a Monte Carlo

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simulated annealing (SA) search were used for optimal conformations of ligands (12). In the latest release (3.0.5), two major features were incorporated into the software. First, addition of two new global search methods: a genetic algorithm (GA) search and an adaptive global-local search method based on Lamarckian Genetics (LGA). Second feature is the addition of an empirical binding free energy force field that allows prediction of binding free energies, hence inhibition constants, for docked ligands (11).

In this study we have used AutoDock 3.0.5 along with its LGA algorithm for automated flexible ligand docking of 32 MMPI-MMP complexes and evaluated docking accuracy and reliability of the estimated inhibition constants.

METHODS

Dataset preparation

Three dimensional coordinates of MMP structures were obtained from the PDB (8). Following criteria were used for the selection of complexes: 1) source organism was human; 2) for crystal structures which were determined by X-ray crystallography, resolution better than 2.6 Å; for structures which were determined by NMR, availability of minimized average structure; 3) availability of the inhibition constant (Ki) for the ligand. Thirty-two metalloproteinase/ligand crystal structures were selected based on the above criteria for the study (table 2). Structures of ligands with variable degree of flexibility (4-18 rotatable bonds; rotors), including their pKi are shown in table 2. HyperChem 7 (13) and AutoDockTools packages were used to prepare docking files.

For each ligand, corresponding ATOM/HETATM and CONECT records were extracted from protein complex in PDB file using a plain text editor. After assigning bond orders, missing hydrogen atoms were added (except for those cases that hydrogen atoms had been assigned by the original authors, namely: 3AYK, 996C, 1HOV, 1FLS, 456C and 830C) and a short minimization (100 steepest descent steps using AMBER95 force fields with a gradient convergence value of 0.05 kcal/mol Å) was performed using HyperChem 7 in order to release any internal strain. Then in the AutoDockTools package, the partial atomic charges were calculated using Gasteiger-Marsili method (14) and after merging non-polar hydrogens, rotatable bonds were assigned. All amide bonds considered as non-rotatable.

For each enzyme, the ligand, as well as any additional chains and all the hetero atoms including water molecules, cofactors (except the zinc ion at the active site) were removed. By the use of AutoDockTools all missing hydrogens were added and after assigning Kollman (15) united atom charges, non-polar hydrogens were merged to their corresponding carbons. Finally, desolvation parameters were assigned to each enzyme atom.

Docking using AutoDock 3.0.5

The grid maps were calculated using AutoGrid (part of the AutoDock package), version 3.06. In all dockings, a grid map with 80 × 80 × 80 points, a grid-point spacing of 0.375 Å (roughly a quarter of the length of a carbon-carbon single bond) were used, and because the location of the ligand in the complex was known, the maps were centered on the ligand's binding site.

Of the three different search algorithms offered by AutoDock 3.0.5, the Lamarckian Genetic Algorithm (LGA) was used, since preliminary experiments using two other methods (SA and GA) showed that they are less efficient than LGA. For all dockings, 50 independent runs with the step sizes of 0.2 Å for translations and 5° for orientations and torsions were performed. For LGA method, an initial population of random individuals with a population size of 50 individuals; a maximum number of 1.5×10^6 energy evaluations; a maximum number of generations of 27000; an elitism value of 1; a mutation rate of 0.02; and a crossover rate of 0.8 were used. In the LGA dockings, the pseudo-Solis and Wets local search method were used. The number of iterations per local search was 300. The possibility of performing local search on an individual in the population was 0.06. The maximum number of consecutive successes or failures before doubling or halving the local search step size was 4 and the termination criterion for the local search was 0.01.

AutoDockTools was used to generate both grid and docking parameter files (i.e. .gpf and .dpf files). The clustering tolerance for the root-mean square positional deviation was 0.5 Å, and the crystallographic coordinates of the ligand were used as the reference structure.

One important problem in carrying out docking studies for MMPs is determination of the zinc ion parameters. Metal ions are modeled in AutoDock by Amber84 force field potentials (15). However, there are no zinc parameters available in the AutoDockTools and it needs zinc radius (r) and well depth (ε) for generation of grid and docking parameter files. One way is using the lj4.py Python script which can be found at AutoDock official site. This script gives r = 1.48 Å and ε = 0.550 kcal/mol for zinc-zinc bond. However in this study Stole-Karplus's (16) parameters (r = 1.10 Å and ε = 0.250 kcal/mol) and a charge of +2.0 e for the zinc ion, were used.

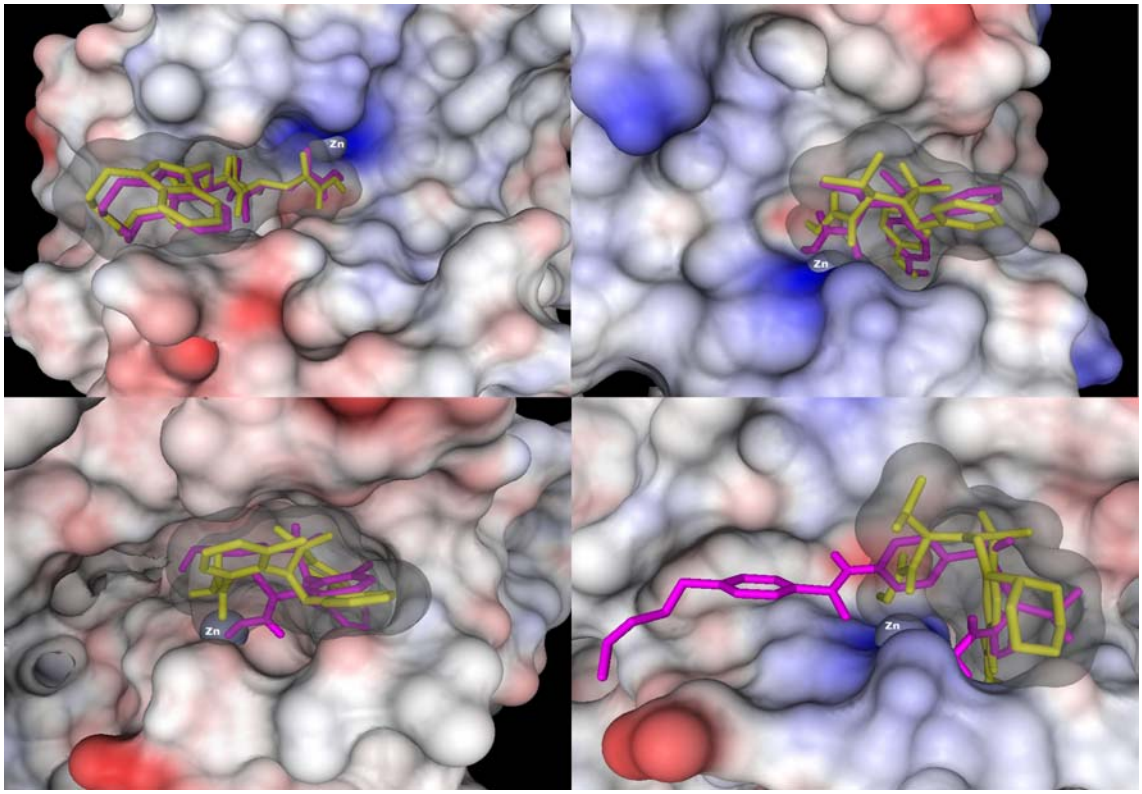


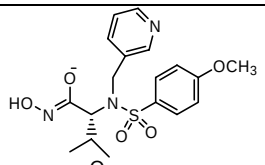
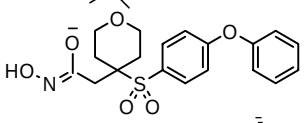
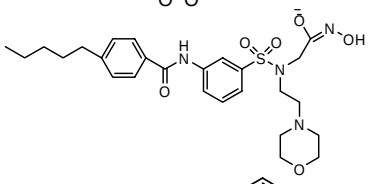
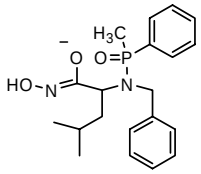
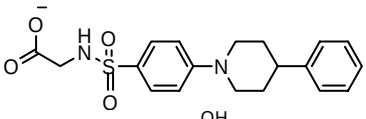
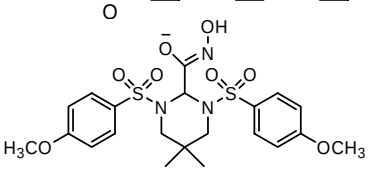
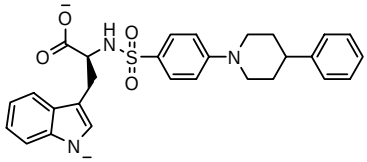
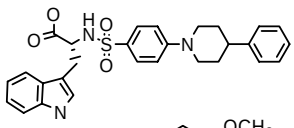
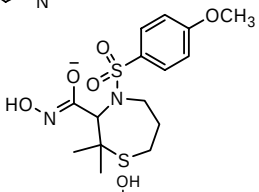
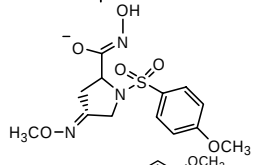
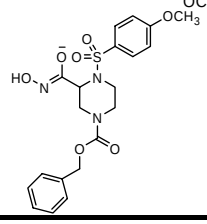
Figure 1. Examples of docking accuracy with excellent, good, fair and poor qualities. The reference structure is shown in yellow and predicted structure in purple. Molecular surface of reference structure is rendered in transparent gray. Top left: the best dock with excellent quality (1MMQ/RRS; RMSD = 0.64 Å). Top right: the best dock with good quality (1JIZ/CGS; RMSD = 1.10 Å). Bottom left: the best dock with fair quality (1FLS/WAY; RMSD = 3.11 Å). Bottom right: the best dock with poor quality (1HOV/SC-74020; RMSD = 5.90 Å).

Table 1. The matrix metalloproteinases

Group	Enzyme	Number	Substrate
I	Interstitial collagenase	MMP-1	Collagen I, II, III
	Neutrophil collagenase	MMP-8	Collagen I, II, III
	Collagenase3	MMP-13	Collagen II
II	Gelatinase A 72 kDA type IV collagenase	MMP-2	Collagen IV, V, VII, X, gelatin, elastin, fibronectin
	Gelatinase B 92 kDA type IV collagenase	MMP-9	Collagen IV, V, VII, X, gelatin, elastin, fibronectin
III	Stromelysin-1	MMP-3	collagen IV, V, IX, laminin proteoglycan, fibronectin
	Stromelysin-2	MMP-10	Collagen IV, V, IX, laminin fibronectin
	Stromelysin-3	MMP-11	α1-Antitrypsin
	Matrilysin	MMP-7	gelatin,fibronectin
IV	Metalloelastase	MMP-12	elastin
V	MT1-MMP	MMP-14	Pro-MMP-2, proteoglycan
	MT2-MMP	MMP-15	Pro-MMP-2, proteoglycan
	MT3-MMP	MMP-16	ND
	MT4-MMP	MMP-17	ND

ND: Not Determined .

Table 2. The 32 MMP/ Ligand complexes used in the docking study

PDB ID	Protein/ligand	Method	Resolution (Å)	Ligand type	pK_i	Rotors	Ligand structure	Refs
3AYK	MMP-1/CGS-27023A	NMR	NA	Hydroxamate	7.31	9		(17)
966C	MMP-1/RS2	X-ray	1.90	Hydroxamate	7.64	7		(18)
1HOV	MMP-2/SC-74020	NMR	NA	Hydroxamate	>10	15		(19)
1B3D	MMP-3/S27	X-ray	2.30	Hydroxamate	7.62	9		(20)
1B8Y	MMP-3/IN7	X-ray	2.00	Carboxylate	7.85	6		(21)
1BQO	MMP-3/PG-117025	X-ray	2.30	Hydroxamate	7.74	8		(22)
1CAQ	MMP-3/DPS	X-ray	1.80	Carboxylate	7.44	7		(21)
1CIZ	MMP-3/DPS	X-ray	1.64	Carboxylate	7.72	7		(21)
1D5J	MMP-3/MM3	X-ray	2.60	Hydroxamate	9.15	5		(23)
1D7X	MMP-3/SPC	X-ray	2.00	Hydroxamate	8.52	7		(24)
1D8F	MMP-3/SPI	X-ray	2.40	Hydroxamate	7.74	8		(25)

NA , Not Applicable.

Table 2 (Continued) . The 32 MMP/ Ligand complexes used in the docking study

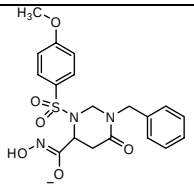
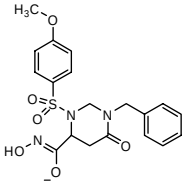
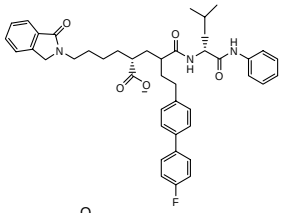
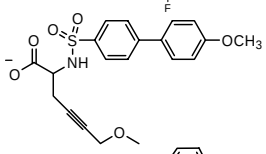
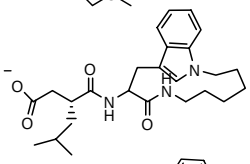
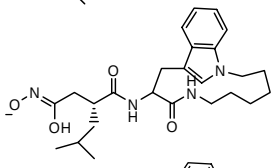
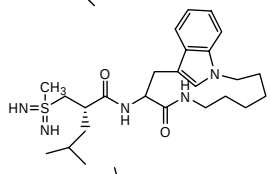
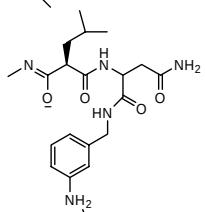
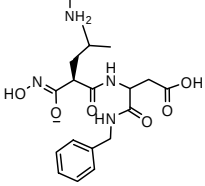
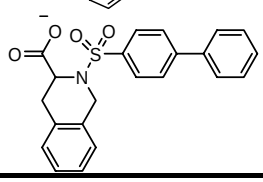
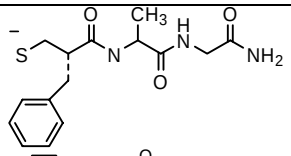
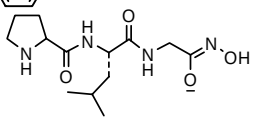
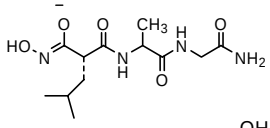
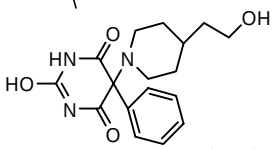
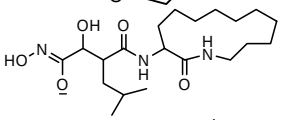
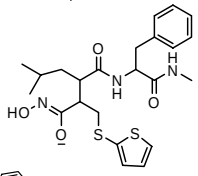
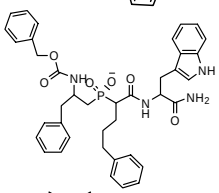
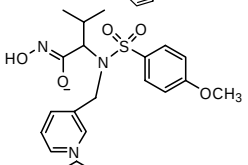
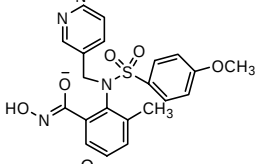
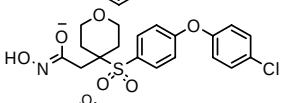
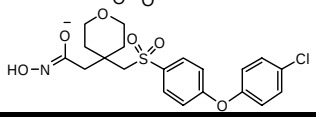
PDB ID	Protein/ ligand	Method	Resolution (Å)	Ligand type	pK_i	Rotors	Ligand structure	Refs
1D8M	MMP-3/BBH	X-ray	2.44	Hydroxamate	8.48	7		(26)
1GO5	MMP-3/BBH	X-ray	2.45	Hydroxamate	8.48	7		(26)
1HFS	MMP-3/LO4	X-ray	1.70	Carboxylate	8.70	18		(27)
1HY7	MMP-3/MBS	X-ray	1.50	Carboxylate	5.39	8		(28)
1MMP	MMP-7/RSS	X-ray	2.30	Carboxylate	6.07	6		(29)
1MMQ	MMP-7/RRS	X-ray	1.90	Hydroxamate	7.52	7		(29)
1MMR	MMP-7/SRS	X-ray	2.30	Sulfodiimine	5.40	6		(29)
1A85	MMP-8/HMI-Asn-BNN	X-ray	2.00	Hydroxamate	4.59	12		(30)
1A86	MMP-8/HMI-Asp-BNN	X-ray	2.00	Hydroxamate	3.87	11		(30)
1BZS	MMP-8/BSI-EPE	X-ray	1.70	Carboxylate	8.00	4		(31)

Table 2 (Continued) . The 32 MMP/ Ligand complexes used in the docking study

PDB ID	Protein/ligand	Method	Resolution (Å)	Ligand type	pK_i	Rotors	Ligand structure	Refs
1JAO	MMP-8/BTP-Asp-GM1	X-ray	2.40	Thiolate	5.92	10		(32)
1JAP	MMP-8/ProLeuGly-HOA	X-ray	1.82	Hydroxamate	4.72	8		(33)
1JAQ	MMP-8/HMP-Asp-GM1	X-ray	2.25	Hydroxamate	4.48	11		(32)
1JJ9	MMP-8/BBT	X-ray	2.00	Barbitorate	5.77	6		(34)
1KBC	MMP-8/HLE-RIN	X-ray	1.80	Hydroxamate	7.70	7		(35)
1MMB	MMP-8/BAT	X-ray	2.10	Hydroxamate	8.30	13		(36)
1HV5	MMP-11/CPS-RXP	X-ray	2.60	Phosphinate	8.05	18		(37)
1JIZ	MMP-12/CGS	X-ray	2.60	Hydroxamate	8.70	9		(38)
1FLS	MMP-13/WAY	NMR	NA	Hydroxamate	7.48	8		(39)
456C	MMP-13/RS-113456	X-ray	2.40	Hydroxamate	9.77	7		(18)
830C	MMP-13/RS-130830	X-ray	1.60	Hydroxamate	9.28	7		(18)

NA , Not Applicable.

Table3 Docking results of 32 MMPIs using AutoDock 3.0.5

PDB ID	Docking		pK_i Prediction			
	RMSD (Å)	Quality[a]	$pKi_{Experimental}$	$pKi_{Predicted}$	$pKi_{Pre} - pKi_{Exp}$	Quality[b]
3AYK	3.28	Fair	7.31	6.92	-[c]	-[c]
966C	0.84	Excellent	7.64	8.76	1.12	Good
1HOV	5.90	Poor	>10	7.95	-[c]	-[c]
1B3D	0.99	Excellent	7.62	7.57	-0.05	Excellent
1B8Y	1.25	Good	7.85	14.88	7.03	Poor
1BQO	0.84	Excellent	7.74	9.06	1.32	Good
1CAQ	0.83	Excellent	7.44	17.09	9.65	Poor
1CIZ	1.61	Good	7.72	16.84	9.12	Poor
1D5J	0.66	Excellent	9.15	8.56	-0.59	Good
1D7X	2.71	Good	8.52	7.58	-0.94	Good
1D8F	3.42	Fair	7.74	8.81	-[c]	-[c]
1D8M	2.35	Good	8.48	8.83	0.35	Excellent
1GO5	1.46	Good	8.48	7.74	-0.74	Good
1HFS	6.43	Poor	8.70	0.00	-[c]	-[c]
1HY7	1.18	Good	5.39	14.02	8.63	Poor
1MMP	0.65	Excellent	6.07	14.37	8.30	Poor
1MMQ	0.64	Excellent	7.52	11.18	3.66	Poor
1MMR	0.90	Excellent	5.40	10.76	5.36	Poor
1A85	1.79	Good	4.59	9.70	5.11	Poor
1A86	2.43	Good	3.87	11.92	8.05	Poor
1BZS	1.26	Good	8.00	13.44	5.44	Poor
1JAO	2.63	Good	5.92	12.69	6.77	Poor
1JAP	7.34	Poor	4.72	6.99	-[c]	-[c]
1JAQ	2.50	Good	4.48	9.73	5.25	Poor
1JJ9	1.28	Good	5.77	7.82	2.05	Fair
1KBC	0.73	Excellent	7.70	9.26	1.56	Fair
1MMB	1.23	Good	8.30	7.46	-0.84	Good
1HV5	2.60	Good	8.05	13.40	5.35	Poor
1JIZ	1.10	Good	8.70	7.10	-1.60	Good
1FLS	3.11	Fair	7.48	7.17	-0.31	Excellent
456C	0.91	Excellent	9.77	8.00	-1.77	Fair
830C	2.52	Good	9.28	7.76	-1.52	Fair

[a] RMSD <1.0 Å = Excellent; 1.0-3.0 Å = Good; 3.0-5.0 Å = Fair; > 5.0 Å = Poor.

[b] $|\Delta pK_i| < 0.5$ = Excellent; 0.5-1.5 = Good; 1.5-2.5 = Fair; > 2.5 = Poor.

[c] Fair and poor dock results were excluded from the reliability test of pK_i prediction.

RESULTS AND DISCUSSION

The results of the LGA docking experiments of the 32 MMP complexes using AutoDock 3.0.5 are summarized in Table. For each docking experiment, the lowest energy docked structure was selected from 50 runs. The CPU time taken for a single docking varied from 62 (1BZS; 4 rotatable bonds) to 205 minutes (1HFS; 18 rotatable bonds), on a 1.4-GHz Pentium 4 machine with 512 MB of RAM and MS Windows XP operating system.

In order to evaluate accuracy of dockings, root-mean square positional deviation (RMSD) of docked ligand from original crystal structure was used. RMSD less than 1.0 Å was considered as excellent, between 1.0-3.0 Å as good, between 3.0-5.0 Å as fair and greater than 5.0 Å as poor (i.e. unacceptable). Examples of the best docking accuracy in each of the four categories are shown in figure 1. Twenty-six out of 32 docks are considered as well-docked with the RMSD less than 3.0 Å. Only three cases (1HOV, 1HFS and 1JAP) failed to dock correctly with RMSD values greater than 5.0 Å which might results from high number of rotatable bonds for the first two cases. Ten cases had RMSD values less than 1.0 Å which was considered as excellent. All the three structures resolved by NMR method (3AYK, 1HOV, 1FLS) had RMSD values greater than 3.0 Å. This may be explained by the fact that AMBER force field which was used by AutoDock was based on X-ray experiments.

Fair and poor dock results were excluded from the reliability test of pKi prediction, because free energy of binding cannot be calculated correctly for incorrect docked geometries. $|\Delta pKi|$ less than 0.5 was considered as excellent, between 0.5-1.5 as good, between 1.5-2.5 as fair and greater than 2.5 as poor. The docking results in Table show that for most of the cases (15 out of 27), predicted pKi values are considerably overestimated in comparison to experimental values (near 10 kcal/mol for two cases). This can be an indication of a problem in binding free energy force field of AutoDock in dealing with metalloproteinases. Although from 30 protein-ligand complexes that were used to calibrate AutoDock's free energy function (11), only one of them was a metalloproteinase (thermolysin), the use of at least one MMP may improve pKi prediction regarding MMPI-MMP complexes.

CONCLUSIONS

Results of this study show that although new wave of flexible ligand docking programs like AutoDock can produce unbiased dockings of inhibitors in enzyme active site in most cases, there is still significant room for improvement especially for the empirical binding free energy force field and inhibition constant prediction. Considering the availability of such a great number of MMPI-MMP complexes in PDB, inclusion of at least one such complex in calibration of the empirical free energy function seems inevitable in the next release of AutoDock.

REFERENCES

1. Murphy G, Docherty AJ. (1992) The matrix metalloproteinases and their inhibitors. Am J Respir Cell Mol Biol. 7**:**120-5.
2. Matrisian LM. (1990) Metalloproteinases and their inhibitors in matrix remodeling. Trends Genet. 6**:**121-5.
3. Murphy G, Hembry RM. (1992) Proteinases in rheumatoid arthritis. J Rheumatol Suppl. 32**:**61-4.
4. Overall CM, Wiebkin OW, Thonard JC. (1987) Demonstration of tissue collagenase activity in vivo and its relationship to inflammation severity in human gingiva. J Periodontal Res. 22**:**81-8.
5. Peress N, Perillo E, Zucker S. (1995) Localization of tissue inhibitor of matrix metalloproteinases in Alzheimer's disease and normal brain. J Neuropathol Exp Neurol. 54**:**16-22.
6. Murphy G, Reynolds JJ, Hembry RM. (1989) Metalloproteinases and cancer invasion and metastasis. Int J Cancer. 44**:**757-60.
7. Becket RP, Davidson AH, Drummond AH, Huxley P, Whittaker M. (1996) Recent advances in matrix metalloproteinase inhibitor research. Drug Discuss. Today. 1**:**16-26.
8. Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, Shindyalov IN, Bourne PE. (2000) The Protein Data Bank. Nucleic Acids Res. 28**:**235-42.
9. Whittaker M, Floyd CD, Brown P, Gearing AJ. (1999) Design and therapeutic application of matrix metalloproteinase inhibitors. Chem Rev. 99**:**2735-76.
10. Abagyan R, Totrov M. (2001) High-throughput docking for lead generation. Curr Opin Chem Biol. 5**:**375-82.
11. Morris GM, Goodsell DS, Halliday RS, Huey R, Hart WE, Belew KR, Olson AJ. (1998) Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. Journal of Computational Chemistry. 19**:**1639-1662.

12. Goodsell DS, Olson AJ. (1990) Automated docking of substrates to proteins by simulated annealing. Proteins: Str. Func. Genet. 8**:**195-202.
13. HyperChem 7. Hypercube, Inc.2002.
14. Gasteiger J, Marsili M. (1980) Iterative partial equalization of orbital electronegativity - A rapid access to atomic charges. Tetrahedron. 36**:**3219-3228.
15. Weiner SJ, Kollman PA, Case DA, Singh UC, Ghio C, Alagona G, Profeta S, Weiner P. (1984) A new force field for molecular mechanical simulation of nucleic acids and proteins. J. Am. Chem. Soc. 106**:**765-784.
16. Stote RH, Karplus M. (1995) Zinc binding in proteins and solution: a simple but accurate nonbonded representation. Proteins. 23**:**12-31.
17. Moy, FJ, Pisano MR, Chanda PK, Urbano C, Killar LM, Sung M.L, Powers R. (1997) Assignments, secondary structure and dynamics of the inhibitor-free catalytic fragment of human fibroblast collagenase. J Biomol NMR. 10**:**9-19.
18. Lovejoy B, Welch AR, Carr S, Luong C, Broka C, Hendricks RT, Campbell JA, Walker K A, Martin R, Van Wart H, Browner MF. (1999) Crystal structures of MMP-1 and -13 reveal the structural basis for selectivity of collagenase inhibitors. Nat Struct Biol. 6**:**217-21.
19. Feng Y, Likos JJ, Zhu L, Woodward H, Munie G, McDonald JJ, Stevens AM, Howard CP, De Crescenzo GA, Welsch D, Shieh HS, Stallings WC. (2002) Solution structure and backbone dynamics of the catalytic domain of matrix metalloproteinase-2 complexed with a hydroxamic acid inhibitor. Biochim Biophys Acta. 1598**:**10-23.
20. Chen L, Rydel TJ, Gu F, Dunaway CM, Pikul S, Dunham KM, Barnett BL. (1999) Crystal structure of the stromelysin catalytic domain at 2.0 A resolution: inhibitor-induced conformational changes. J Mol Biol. 293**:**545-57.
21. Pavlovsky AG, Williams MG, Ye QZ, Ortwine DF, Purchase CF, White, AD, Dhanaraj V, Roth BD, Johnson LL, Hupe D, Humblet C, Blundell TL. (1999) X-ray structure of human stromelysin catalytic domain complexed with nonpeptide inhibitors: implications for inhibitor selectivity. Protein Sci. 8**:**1455-62.
22. Pikul S, McDow Dunham KL, Almstead NG, De B, Natchus MG, Anastasio MV, McPhail SJ, Snider CE, Taiwo YO, Rydel T, Dunaway CM, Gu F, Mieling GE. (1998) Discovery of potent, achiral matrix metalloproteinase inhibitors. J Med Chem. 41**:**3568-71.
23. Almstead NG, Bradley RS, Pikul S, De B, Natchus MG, Taiwo YO, Gu F, Williams LE, Hynd BA, Janusz MJ, Dunaway CM, Mieling GE. (1999) Design, synthesis, and biological evaluation of potent thiazine- and thiazepine-based matrix metalloproteinase inhibitors. J Med Chem. 42**:**4547-62.
24. Cheng M, De B, Almstead NG, Pikul S, Dowty ME, Dietsch CR, Dunaway CM, Gu F, Hsieh LC, Janusz MJ, Taiwo YO, Natchus MG, Hudlicky T, Mandel M. (1999) Design, synthesis, and biological evaluation of matrix metalloproteinase inhibitors derived from a modified proline scaffold. J Med Chem. 42**:**5426-36.
25. Cheng M, De B, Pikul S, Almstead NG, Natchus MG, Anastasio MV, McPhail SJ, Snider C E, Taiwo YO, Chen L, Dunaway CM, Gu F, Dowty ME, Mieling GE, Janusz MJ, Wang-Weigand S. (2000) Design and synthesis of piperazine-based matrix metalloproteinase inhibitors. J Med Chem. 43**:**369-80.
26. Pikul S, Dunham KM, Almstead NG, De B, Natchus MG, Taiwo YO, Williams LE, Hynd BA, Hsieh LC, Janusz MJ, Gu F, Mieling GE. (2001) Heterocycle-based MMP inhibitors with P2' substituents. Bioorg Med Chem Lett. 11**:**1009-13.
27. Esser CK, Bugianesi RL, Caldwell CG, Chapman KT, Durette PL, Girotra NN, Kopka IE, Lanza TJ, Levorse DA, MacCoss M, Owens KA, Ponpipom MM, Simeone JP, Harrison R K, Niedzwiecki L, Becker JW, Marcy AI, Axel MG, Christen AJ, McDonnell J, Moore VL, Olszewski JM, Saphos C, Visco DM, Hagmann WK. (1997) Inhibition of stromelysin-1 (MMP-3) by P1'-biphenylylethyl carboxyalkyl dipeptides. J Med Chem. 40**:**1026-40.
28. Natchus MG, Bookland RG, Laufersweiler MJ, Pikul S, Almstead NG, De B, Janusz MJ, Hsieh LC, Gu F, Pokross ME, Patel VS, Garver SM, Peng SX, Branch TM, King SL, Baker TR, Foltz DJ, Mieling GE. (2001) Development of new carboxylic acid-based MMP inhibitors derived from functionalized propargylglycines. J Med Chem.; 44**:**1060-71.
29. Browner MF, Smith WW, Castelhano AL. (1995) Matrilysin-inhibitor complexes: common themes among metalloproteases. Biochemistry. 34**:**6602-10.
30. Brandstetter H, Engh RA, Von Roedern EG, Moroder L, Huber R, Bode W, Grams F. (1998) Structure of malonic acid-based inhibitors bound to human neutrophil collagenase. A new binding mode explains apparently anomalous data. Protein Sci. 7**:**1303-9.

31. Matter H, Schwab W, Barbier D, Billen G, Haase B, Neises B, Schudok M, Thorwart W, Schreuder H, Brachvogel V, Lonze P, Weithmann KU. (1999) Quantitative structure-activity relationship of human neutrophil collagenase (MMP-8) inhibitors using comparative molecular field analysis and X-ray structure analysis. J Med Chem. 42**:**1908-20.
32. Grams F, Reinemer P, Powers JC, Kleine T, Pieper M, Tschesche H, Huber R, Bode W. (1995) X-ray structures of human neutrophil collagenase complexed with peptide hydroxamate and peptide thiol inhibitors. Implications for substrate binding and rational drug design. Eur J Biochem. 228**:**830-41.
33. Bode W, Reinemer P, Huber R, Kleine T, Schnierer S, Tschesche H. (1994) The X-ray crystal structure of the catalytic domain of human neutrophil collagenase inhibited by a substrate analogue reveals the essentials for catalysis and specificity. Embo J. 13**:**1263-9.
34. Brandstetter H, Grams F, Glitz D, Lang A, Huber R, Bode W, Krell HW, Engh RA. (2001) The 1.8-A crystal structure of a matrix metalloproteinase 8-barbiturate inhibitor complex reveals a previously unobserved mechanism for collagenase substrate recognition. J Biol Chem. 276**:**17405-12.
35. Betz M, Huxley P, Davies SJ, Mushtaq Y, Pieper M, Tschesche H, Bode W, Gomis-Ruth F X. (1997) 1.8-A crystal structure of the catalytic domain of human neutrophil collagenase (matrix metalloproteinase-8) complexed with a peptidomimetic hydroxamate primed-side inhibitor with a distinct selectivity profile. Eur J Biochem. 247**:**356-63.
36. Grams F, Crimmin M, Hinnes L, Huxley P, Pieper M, Tschesche H, Bode W. (1995) Structure determination and analysis of human neutrophil collagenase complexed with a hydroxamate inhibitor. Biochemistry. 34**:**14012-20.
37. Gall AL, Ruff M, Kannan R, Cuniasse P, Yiotakis A, Dive V, Rio MC, Basset P, Moras D. (2001) Crystal structure of the stromelysin-3 (MMP-11) catalytic domain complexed with a phosphinic inhibitor mimicking the transition-state. J Mol Biol. 307**:**577-86.
38. Nar H, Werle K, Bauer MM, Dollinger H, Jung B. (2001) Crystal structure of human macrophage elastase (MMP-12) in complex with a hydroxamic acid inhibitor. J Mol Biol. 312**:**743-51.
39. Moy FJ, Chanda PK, Chen JM, Cosmi S, Edris W, Levin JI, Powers R. (2000) High-resolution solution structure of the catalytic fragment of human collagenase-3 (MMP-13) complexed with a hydroxamic acid inhibitor. J Mol Biol 302**:**671-89.