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Discovery of novel human acrosin inhibitors by virtual screening

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Abstract Human acrosin is an attractive target for the discovery of male contraceptive drugs. For the first time, structure-based drug design was applied to discover structurally diverse human acrosin inhibitors. A parallel virtual screening strategy in combination with pharmacophore-based and docking-based techniques was used to screen the SPECS database. From 16 compounds selected by virtual screening, a total of 10 compounds were found to be human acrosin inhibitors. Compound **2** was found to be the most potent hit ($IC_{50} = 14$ μM) and its binding mode was investigated by molecular dynamics simulations. The hit interacted with human acrosin mainly through hydrophobic and hydrogen-bonding interactions, which provided a good starting structure for further optimization studies.

Keywords Human acrosin · Virtual screening · Male contraceptives · Lead structure

Introduction

The control of human fertility is an important global issue because overpopulation and unintended pregnancy have personal and social impacts. Since the 1960s, the development of hormonal-based oral contraceptives for women established an effective and relatively safe way of preventing pregnancies. Today, ~55 million women use oral contraceptives, which is the most popular form of contraception. In contrast, the development of a male-based contraceptive has lagged far behind. The discovery of an effective, safe and reversible male contraceptive remains a challenge.

Human acrosin, a serine protease located in the epididymis, has an important role in fertilization [1]. Recent studies revealed that human acrosin has multiple functions during fertilization, such as acrosomal exocytosis, as well as acting as a receptor for zona pellucida proteins and as a protease to facilitate penetration of spermatozoa into the egg [2]. The special location and unusually high cell specificity of human acrosin has made it a potential target for developing novel male contraceptive agents. Several classes of human acrosin inhibitors have been reported, including bdellins [3], *N*-alpha-tosyl-*L*-lysyl-chloromethylketone (TLCK) [4], suramin [5], *p*-aminobenzamidine (pAB) [6], isoxazolecarbaldehydes [7], and 4-guanidinobenzoates [8] (Fig. 1). However, human acrosin inhibitors have several drawbacks such as low affinity, lack of selectivity and high toxicity. An effective way to overcome these problems is to design specific and highly potent inhibitors on the basis of the three-dimensional (3D) structural information of human acrosin.

In 2000, Tranter et al. [9] solved the crystal structures of ram and boar β-acrosins in complex with *p*-aminobenzamidine. However, the crystal structure of human β-acrosin has not been reported until now. We previously constructed a 3D model of human β-acrosin through homology modeling on the basis of the crystallographic coordinates of ram and boar β-acrosins [10]. Moreover, the active site of human acrosin was analyzed by the multiple copy

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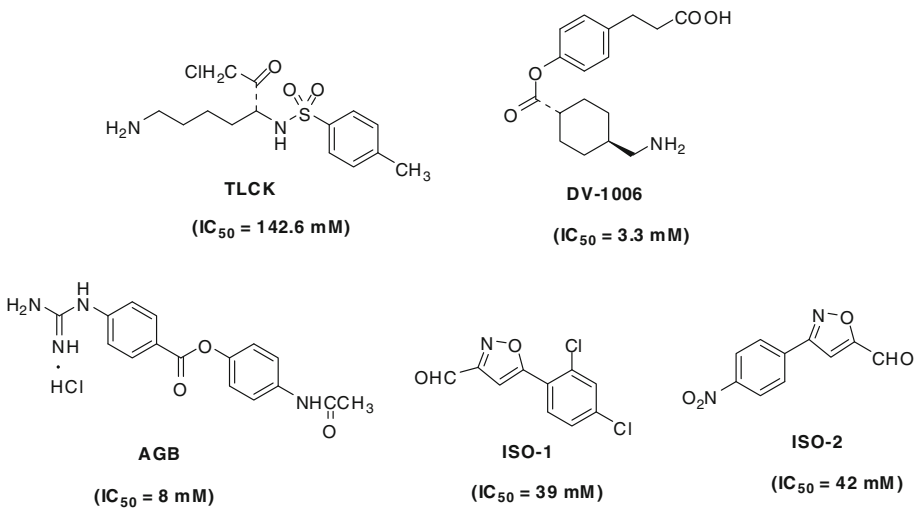

Fig. 1 Chemical structures of representative human acrosin inhibitors

simultaneous search (MCSS) method, and the binding mode of known inhibitors investigated by molecular docking [11].

Virtual screening is becoming a powerful tool for hit and lead discovery [12–14]. In this work, we describe the rapid and successful identification of novel acrosin inhibitors using a parallel strategy of pharmacophore-based and docking-based virtual screening. Ten hits were identified to possess acrosin inhibitory activities. To the best of our knowledge, this is the first example of the successful application of virtual screening to discover novel acrosin inhibitors.

Results and discussion

Structure-based pharmacophore generation and model validation

Pharmacophore-based methods have been widely used in virtual screening [15]. Structure-based pharmacophore generation uses the spatial information of the target protein for topological description of ligand–receptor interactions. It also provides an efficient alternative to docking-based virtual screening while continuing to represent specific ligand–protein interactions. Moreover, recent studies indicated that the structure-based pharmacophore approach provided more detailed information and accuracy in its description of ligand binding than ligand-based methods [16]. Because there are few potent acrosin inhibitors, a structure-based pharmacophore was generated using the "Interaction Generation" module of Discovery Studio 2.5 (DS 2.5) software package [17] (Fig. 2). The resulting pharmacophoric features were clustered into two hydrogen-bond acceptors (HA), one hydrogen-bond donor (HD) and two hydrophobic regions (HP). In addition, the excluded volumes were involved in the pharmacophore models to improve the effectiveness of virtual screening. The pharmacophore model was consistent with the MCSS results of the active site of human acrosin [11]. It was demonstrated that the active site of human acrosin could be divided into three pockets: P1, P2 and G. The P1 pocket was a hydrophobic hydrogen-bonding site for inhibitor binding. Two HAs were found to be located in this region, which corresponded to Thr216 and Ser221, respectively. The hydrophobic regions were mainly distributed on the edge of the P1 and P2 pockets, and two HPs were located in these two pockets accordingly. One HD element

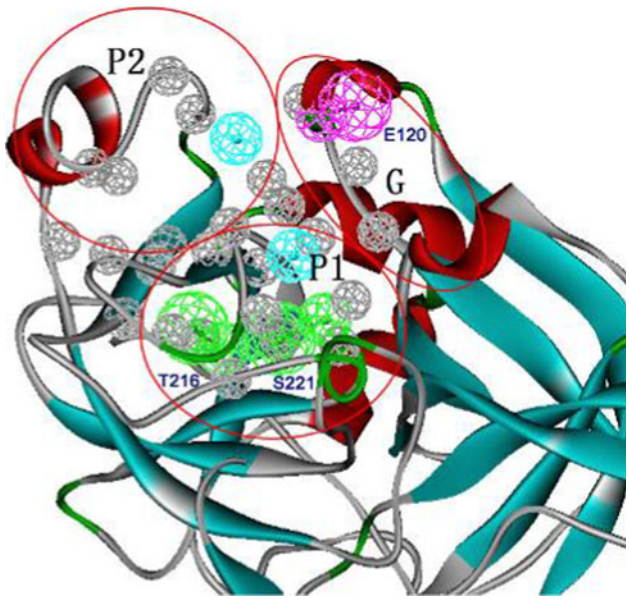

Fig. 2 Structure-based pharmacophore model of human acrosin including two HAs (*green spheres*), one HD (*magenta sphere*), two HPs (*cyan spheres*) and 25 exclusion spheres (*gray spheres*)

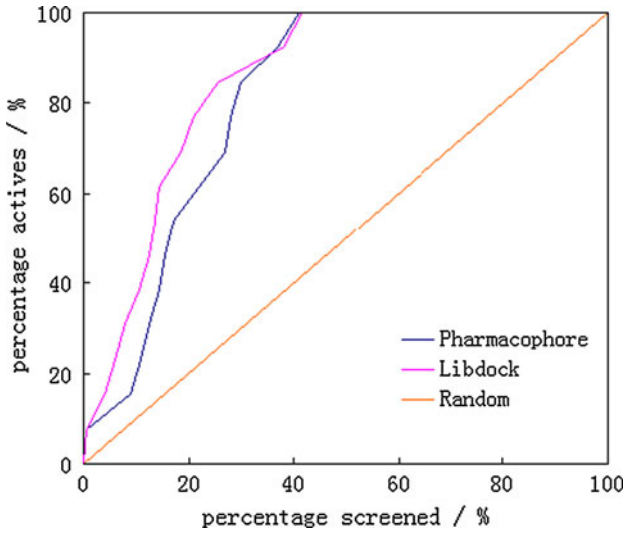

Fig. 3 Enrichment factors of human acrosin inhibitors obtained from pharmacophore-based (*magenta*) and docking-based (*blue*) virtual screening tests

corresponding to Glu120 was located in pocket G, which was identified as another hydrogen-bonding site.

To validate the pharmacophore model and demonstrate its efficiency, a virtual screening test was undertaken to ascertain whether or not it could differentiate the known acrosin inhibitors from the "drug-like" decoy ligands. We constructed a test set containing 187 drug-like molecules extracted from the MDL Drug Data Report (MDDR) dataset and 13 known acrosin inhibitors with different scaffolds (dataset can be found in Supplementary Material). The enrichment factor (EF) was plotted, and represented the fraction of the database that was screened versus the percentage of the active compound found (Fig. 3). When the top 20 and 40% of the scored dataset was analyzed, 55 and 100% of the acrosin inhibitors were identified. This demonstrated the quality of the pharmacophore hypotheses.

Validation of the docking method

The reliability of various kinds of docking approaches was evaluated to discern the best docking method for virtual screening. Following a well-accepted approach [18], *p*-aminobenzamidine were docked into the crystal structure of boar acrosin (PDB code: 1FIZ) through LibDock [19], Gold 3.0.1 [20], and Autodock 3.0 [21], respectively. The docking reliability was evaluated through a comparison of the root-mean-square deviation (RMSD) between the positions of the heavy atoms of the ligand in the calculated and experimental structures. The calculated RMSD value for the three docking methods was 0.45 Å, 1.24 Å and 0.67 Å, respectively, suggesting that LibDock gave the best results. Furthermore, LibDock was validated by the virtual

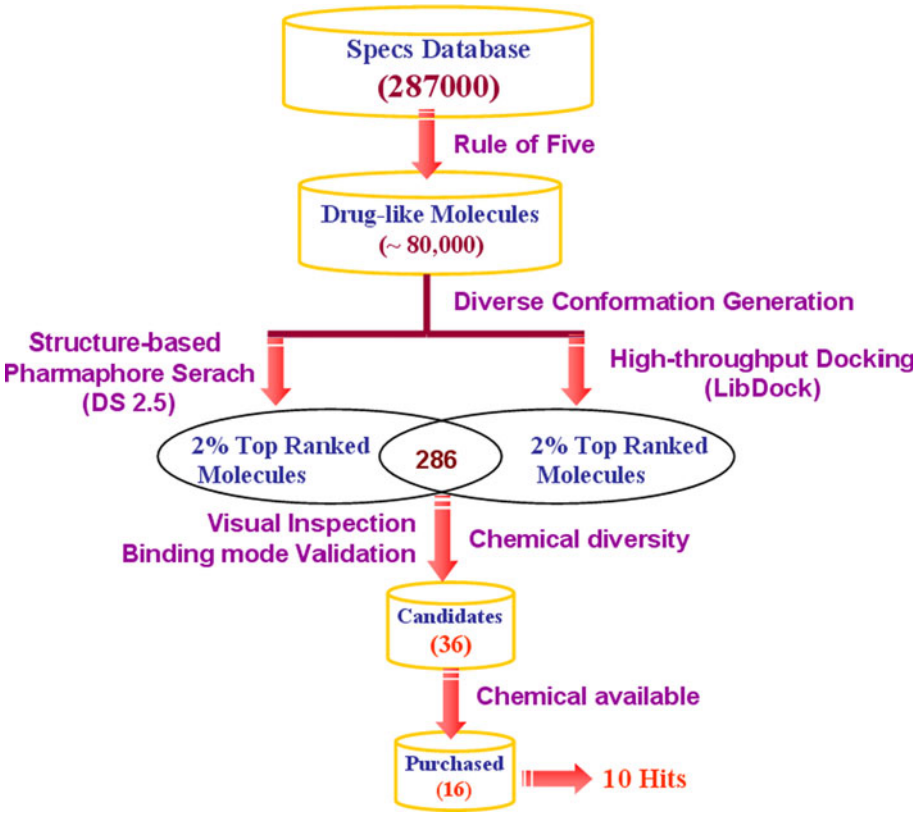

Fig. 4 Flowchart of the parallel virtual screening procedure

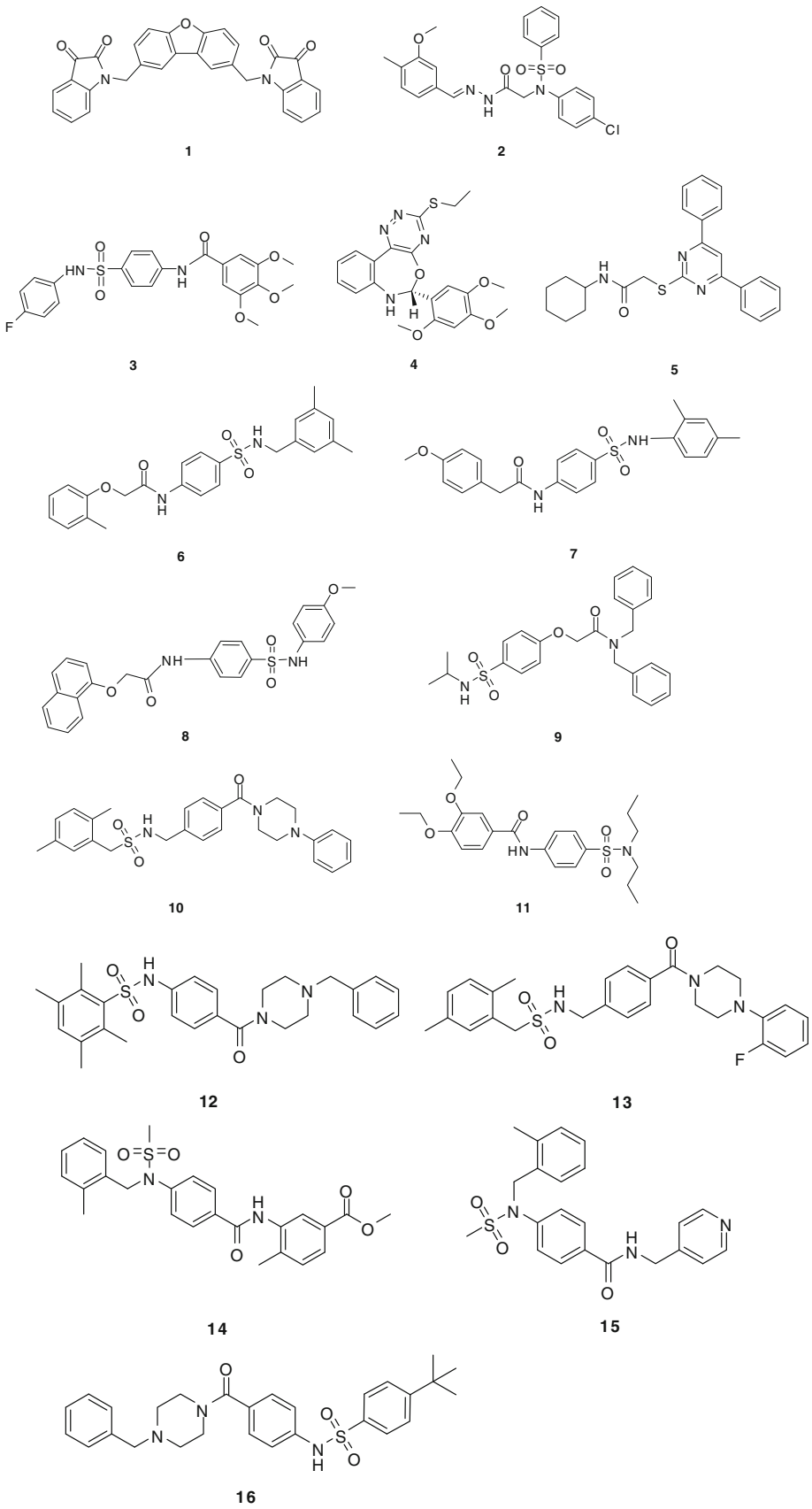

Fig. 5 Chemical structures of the purchased compounds from virtual screening

Table 1 Physical properties, scores and human acrosin inhibitory activities of hits from virtual screening

Compound	MW	ALogP[a]	LibDock score	Fit value	IC_{50} (μM)
2	472.96	4.259	133.0835	2.759	14.0 ± 0.69
3	461.48	3.278	130.395	2.681	205.3 ± 3.7
5	405.56	4.724	141.499	2.916	355.1 ± 8.4
8	463.53	3.849	133.883	2.569	667.8 ± 10.8
9	453.57	3.780	130.18	2.667	426.7 ± 6.1
11	449.58	4.037	136.075	2.491	301.8 ± 9.7
12	492.65	3.656	133.6775	2.574	624.8 ± 11.6
14	467.56	4.039	138.5790	2.475	155.5 ± 5.3
15	410.51	2.554	135.793	2.399	344.7 ± 7.1
16	492.65	3.112	146.4319	2.469	3056.8 ± 14.9
TLCK	332.846	1.746	100.124	2.930	142.6 ± 4.1[b]

[a] AlogP values were calculated using the Discovery Studio 2.5 software package; [b] unit is mM

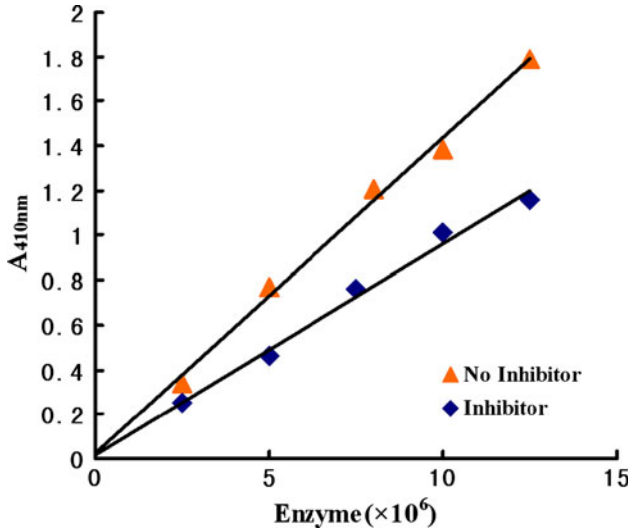

Fig. 6 Variation of optical density (OD) values at 410 nm wavelength with respect to the concentration of human acrosin. (*filled triangle*) Control sample containing BAPNA (1.86 mM) (*filled diamond*) Test sample containing BAPNA (1.86 mM) and hit **2** (1.86 mM). The control sample and test sample showed a good linear relationship. They intersected at the origin, and the test sample had a lower slope, indicating that hit 2 was a reversible inhibitor of human acrosin

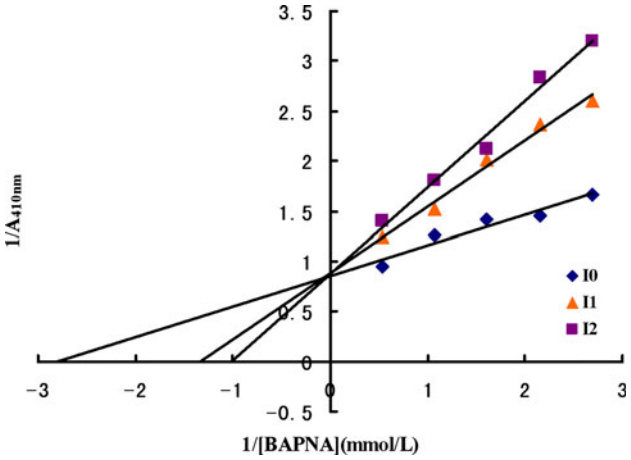

Fig. 7 Lineweaver-Burk plot of the kinetics of human acrosin. (*filled diamond*) I0, control sample containing BAPNA; (*filled triangle*) I1, test sample containing hit **2** (14 μM) and BAPNA; (*filled square*) I2, test sample containing hit **2** (28 μM) and BAPNA. The control sample and test sample showed a good linear relationships. They intersected at the *y* axis, indicating that hit **2** was a competitive inhibitor of human acrosin

screening test described above. Figure 3 indicates that the effectiveness of LibDock in virtual screening was slightly better than that of the pharmacophore-based method. When the top 20 and 40% of the scored dataset was analyzed, 65 and 100% of the acrosin inhibitors were identified. Therefore, LibDock could be a suitable method for structure-based virtual screening of human acrosin inhibitors.

Virtual screening

Taking advantage of complementary pharmacophore-based and docking-based screening techniques and overcoming their limitations, we searched the SPECS database (www.specs.com) via a parallel virtual screening strategy. A schematic summary of the overall virtual screening procedure in this study is presented in Fig. 4. First, Lipinski's rule-of-five was used to filter-out compounds which had poor drug-like properties. To incorporate ligand flexibility in virtual screening, diverse conformations of resulting molecules were generated by the conformation module within the DS 2.5 software package. On the basis of these conformations, parallel pharmacophore-based and docking-based virtual screening was carried out. All docking and pharmacophore mapping conformations were ranked by their scoring functions. Finally, 286 molecules were found to be located at the intersection point from the 2%

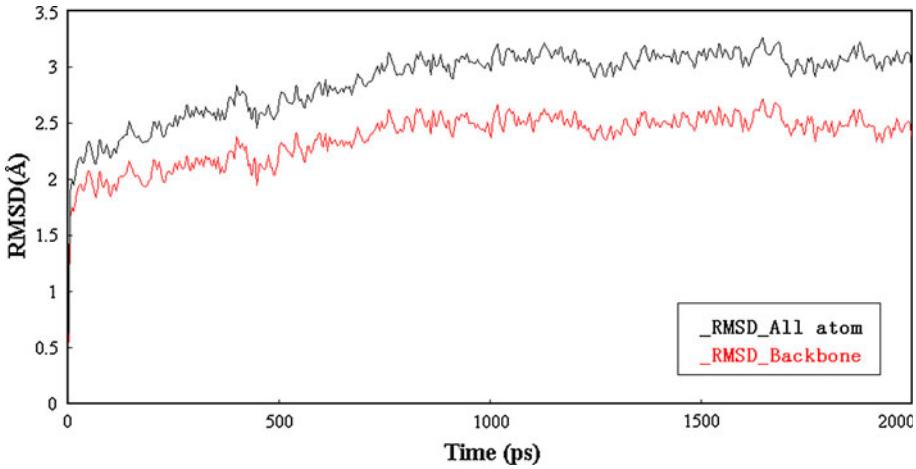

Fig. 8 RMSD values of the backbone α-atoms (*black*) and all atoms (*red*) of the protein with respect to the initial equilibrated configuration as a function of time

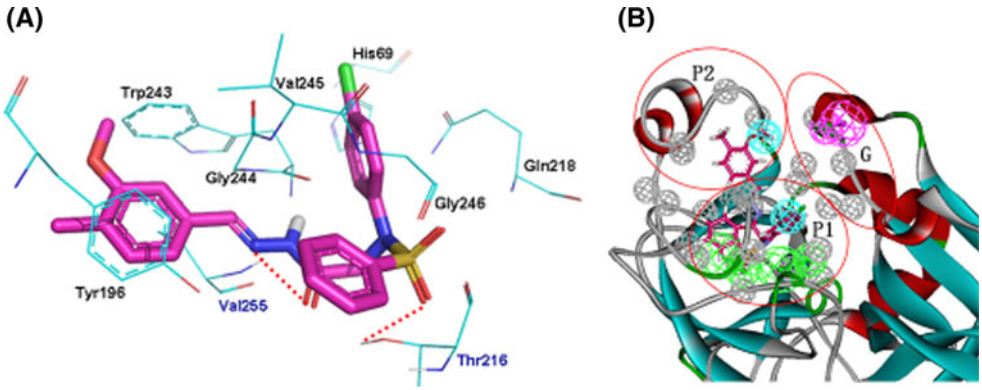

Fig. 9 **a** The binding mode of hit **2** in the active site of human acrosin; **b** hit **2** aligned with the structure-based pharmacophore hypothesis

top-ranked molecules of pharmacophore-based and docking-based scores.

Compound selection and in vitro inhibitory activities of human acrosin

The selection of candidate molecules was based on four criteria. The first criterion was a reasonable chemical structure and pose in the active site of human acrosin. Some unusually highly scored molecules were found to have many rotatable bonds (e.g., long aliphatic structures), which were excluded for further evaluation. The second criterion was formation of at least one hydrogen bond between the ligand and the important residues of human amino-acid residues (e.g., Thr216). The third criterion was that the binding mode of the compounds could be reproduced by at least two docking methods. The final criterion was that the chemical diversity of candidate molecules was taken into account. For compounds with the same scaffold, only the compound with the highest score was kept. As a result, 36 candidate compounds met the criteria mentioned above and were designated for purchase. However, only 16 compounds were found to be commercially available for in vitro assay (Fig. 5).

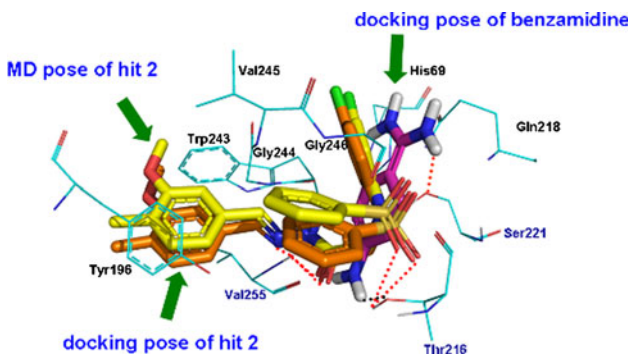

Fig. 10 Comparison of the binding mode of hit 2 (*yellow* for the MD pose and *orange* for the docking pose) and benzamidine (colored by atom type). Hydrogen bonds are displayed as *red dotted lines*

The human acrosin inhibitory activities of the purchased compounds were evaluated in an in vitro assay using TLCK as a standard reference drug. The results of the assay (along with pharmacophore-fit values and Libdock scores) are shown in Table 1. Of the 16 compounds tested, 10 were found to be inhibitors of human acrosin with a spectrum of half maximal inhibitory concentration (IC_{50}) values ranging from 14 μM to 3.06 mM (Table 1). For most of the hits, their activities were on a level similar to those of the reported human acrosin inhibitors. In particular, compound

2 showed the highest inhibitory activity (IC_{50} = 14 μM), which provided a good starting point for further optimization studies. To investigate the selectivity of hit **2**, we determined its inhibitory activity against human trypsin (a member of the serine protease family). The IC_{50} value was >1.5 mM, indicating that hit **2** exhibited good specificity toward human acrosin. Moreover, we studied the inhibitory mechanism of hit **2** by well-accepted methods [22, 23]. Hit **2** was found to be a reversible and competitive inhibitor of human acrosin (Figs. 6, 7).

The binding mode of the new human acrosin inhibitor

To obtain the accurate binding mode of the hits, molecular dynamics (MD) simulations were undertaken on the docking model of compound **2** (the most potent hit). We used the Desmond method (http://www.deshawresearch.com) to carry out all the simulations. After 2-ns MD calculations, a stable complex was obtained. The structures of the complex deviated rapidly from the initial docked configuration within the first 800 ps of the MD simulation (Fig. 8). After ~1 ns, the total RMSD was stabilized under 3 Å, suggesting that the complex was stable during the MD simulation. The MD results indicated that the conformation of compound **2** in the active site of human acrosin was very similar to that in the docking model (RMSD < 2 Å) and that important hydrophobic and hydrogen-bonding interactions were preserved.

Compound **2** bound to the active site of human acrosin mainly through hydrophobic and hydrogen-bonding interactions (Fig. 9a). The methyl-substituted benzene ring was extended to the P2 pocket, which was surrounded by hydrophobic residues lined with Tyr196, Val245, Gly244 and Trp243. The bensulfamide group was located in the P1 pocket, and its oxygen atom formed a hydrogen bond with the hydroxyl group of the key residue Thr216. Moreover, the amide oxygen atom and hydrazine nitrogen atom could form two hydrogen bonds with the backbone NH of Val255. Another benzene ring was located into the edge of the P1 pocket and formed hydrophobic interactions with Val245, Gln218 and Gly246. Compound **2** also matched well with the structure-based pharmacophore model (Fig. 9b). It was shown that its methoxy group and chlorine-substituted benzene ring was matched with two HPs, and its oxygen atom of sulfanilamine matched one HA in the P1 pocket. Furthermore, hit **2** was docked into the active site of ram and boar acrosin to compare the difference in key interactions. The results indicated that the hydrogen-bonding interactions were not conserved (Figure 1 in Supplementary Material). The oxygen atom of the bensulfamide group and the hydrazine amine group of hit **2** formed hydrogen bonds with Gln 192 and Gly216 (corresponding to Ile214 and Val238 in human acrosin) of ram and boar acrosin, respectively. Therefore, it was necessary to use the homology model to carry out virtual screening studies instead of using the crystal structure of ram or boar acrosin.

The hits shared low molecular similarity with previously known acrosin inhibitors. In particular, they lacked a positively charged group that is common in many serine protease inhibitors (e.g., *p*-aminobenzamidine). We previously investigated the binding mode of known acrosin inhibitors [11]. In most cases, their scaffold and positively charged group were located in the P1 pocket. Their positively charged group could form hydrogen-bonding interactions with Ser221, a key residue of the catalytic triad in human acrosin [9]. For example, *p*-aminobenzamidine interacted with the P1 pocket through its amidine and amine groups to form hydrogen bonds with Ser221 and Thr216, respectively. A comparison of the binding mode of hit **2** and *p*-aminobenzamidine is depicted in Fig. 10. It was observed that hit **2** did not interact with Ser221 (another HA element in the P1 pocket) and Glu210 (HD element in the G pocket) directly. This suggested that the addition of these interactions would significantly improve the inhibitory activity.

Conclusion

Human acrosin is a promising target in the discovery of male contraceptive drugs. Structurally diverse human acrosin inhibitors were obtained by a parallel virtual screening strategy in combination with pharmacophore-based and docking-based methods. Sixteen compounds were initially selected for in vitro testing, 10 of which were found to have inhibitory activity in an enzyme assay. They had little structural similarity to other known acrosin inhibitors. In particular, the IC_{50} value of hit **2** was 14 μM, highlighting the importance for further structure–activity relationship studies. The binding mode of hit **2** obtained by MD simulations provided a structural basis for further optimization studies.

Experimental section

Structure-based pharmacophore model generation

The 3D structure of human acrosin was obtained from our previous studies [10]. According to the homologous 3D model of human acrosin and the properties of the active site, 1,062 features, including hydrogen donors (HD), hydrogen acceptors (HA) and hydrophobic regions (HP), were generated through the Interaction Generation protocol in the DS 2.5 software package. These features were clustered into one HA, one HD and two HPs by the Unweighted Pair Group Method with Arithmetic Mean

(UPGMA) algorithm. In addition, the excluded volumes were involved in the pharmacophore models to improve the effectiveness of virtual screening [24–26].

Virtual screening

The virtual screening flowchart in Fig. 4 was constructed by Pipeline Pilot 7.5 within the DS 2.5 software package. First, the filter of Lipinski's rule of five was used to select drug-like compounds from the SPECS database. Then, the diverse conformations of the resulting molecules were generated by the "Diverse Conformation Generator" module with the conformation method set to fast and the maximum conformations set to 255. Subsequently, each molecule was scored and ranked by the structure-based pharmacophore hypothesis and Libdock, respectively. The pharmacophore model screen was carried out by using a ligand pharmacophore map in DS 2.5 with the maximum omitted features set to 4 and the fitting method set to rigid. In the process of Libdock docking, the number of hotspots was set to 250 and the docking preferences parameter set to fast search. Lastly, the intersection point from the 2% top-ranked molecules of pharmacophore-based and docking-based scores was subjected to visual inspection, binding mode analyses and chemical diversity analyses to determine the finally purchased compounds.

MD simulations

The Desmond_Maestro_academic-2009-02 version of Desmond [27–30] with the OPLS-AA force field was used to carry out all the simulations. The docking model of the hit **2**–human acrosin complex was neutralized by adding the salt counterion and the concentration set to 0.15 using a system builder package. As a result, the complex was immersed in an orthorhombic box containing TIP3P water molecules. The cutoff distance for the non-bonded interactions was 9 Å, and periodic boundary conditions were applied. Electrostatic interactions were represented using the Smooth Particle Mesh Ewald method with a grid spacing of 10 Å. The systems were then simulated for 2 ns at 300 K and 1 atm. System coordinates were saved every 4.8 ps for further analyses and heavy-atom RMSD from the energy-minimized model structure was calculated for each MD snapshot [31, 32].

Biological assay

The acrosin inhibitory activities of the purchased compound were determined by the method of Kennedy et al. [33]. Briefly, 0.1 mL of liquefied human semen was carefully layered over 0.5 mL of 11% Ficoll solution containing NaCl (0.12 mol/L) and HEPES buffer (pH = 7.4, 0.025 mol/L) in a conical centrifuge tube and centrifuged at 1,000 g for 30 min. After carefully removing the seminal plasma and Ficoll solution without disturbing the sperm pellet, 0.1 mL of test compound solution (0.1 mL of buffer in control tubes) was added and mixed. TLCK was used as the positive control. Finally, 0.8 mL of buffer–substrate solution containing *N*-α-benzoyl-L-arginine *p*-nitroanilide (BAPNA, 0.1%), Triton X-100 (0.01%), HEPES buffer (0.055 mol/L) and NaCl (0.055 mol/L) at pH 8.0 was added, mixed thoroughly and incubated at 37.8 °C for 3 h. At the end of the incubation period, 100 mL of benzamidine hydrochloride solution (0.5 mol/L) was added to stop the reaction and the tubes centrifuged at 1,000 g for 30 min. The optical density (OD) of the supernatant was read at 410 nm in a spectrophotometer. The inhibition activities were calculated according to the OD values. The inhibitory activity of hit **2** against human trypsin [34] and its kinetic effects on human acrosin [35] were determined by a similar procedure.

References

1. Klemm U, Muller-Esterl W, Engel W (1991) Acrosin, the peculiar sperm-specific serine protease. Hum Genet 87:635–641
2. Howes L, Jones R (2002) Interactions between zona pellucida glycoproteins and sperm proacrosin/acrosin during fertilization. J Reprod Immunol 53:181–192
3. Baskova IP, Zavalova LL (2001) Proteinase inhibitors from the medicinal leech Hirudo medicinalis. Biochemistry (Mosc) 66:703–714
4. Pakzad R (1989) The effect of the trypsin inhibitor aprotinin (Trasylol) and TLCK on the gelatinolytic activity of acrosin and the motility of rabbit sperm in vitro. Z Mikrosk Anat Forsch 103:8–13
5. Jones R, Parry R, Lo Leggio L, Nickel P (1996) Inhibition of sperm-zona binding by suramin, a potential 'lead' compound for design of new anti-fertility agents. Mol Hum Reprod 2:597–605
6. Fraser LR (1982) p-Aminobenzamidine, an acrosin inhibitor, inhibits mouse sperm penetration of the zona pellucida but not the acrosome reaction. J Reprod Fertil 65:185–194
7. Gupta G, Jain RK, Maikhuri JP, Shukla PK, Kumar M, Roy AK, Patra A, Singh V, Batra S (2005) Discovery of substituted isoxazolecarbaldehydes as potent spermicides, acrosin inhibitors and mild anti-fungal agents. Hum Reprod 20:2301–2308
8. Bourinbaiar AS, Lee-Huang S (1995) Acrosin inhibitor, 4′-acetamidophenyl 4-guanidinobenzoate, an experimental vaginal contraceptive with anti-HIV activity. Contraception 51:319–322
9. Tranter R, Read JA, Jones R, Brady RL (2000) Effector sites in the three-dimensional structure of mammalian sperm beta-acrosin. Structure 8:1179–1188
10. Lu JG, Sheng CQ, Zhang M, Ji HT, Zhang WN, Zhou YJ, Zhu J, Jiang JH (2006) Homology modeling of human acrosin and its molecular docking study with KF950. Acta Chimica Sinica 64:1073–1078
11. Zhang J, Zheng C, Sheng C, Zhou Y, Zhu J, Lv J (2009) Characterization of human acrosin active site and binding modes with its inhibitors. Chem J Chinese U 30:2409–2414
12. Kolb P, Rosenbaum DM, Irwin JJ, Fung JJ, Kobilka BK, Shoichet BK (2009) Structure-based discovery of beta2-adrenergic receptor ligands. Proc Natl Acad Sci U S A 106:6843–6848

13. Li H, Huang J, Chen L, Liu X, Chen T, Zhu J, Lu W, Shen X, Li J, Hilgenfeld R, Jiang H (2009) Identification of novel falcipain-2 inhibitors as potential antimalarial agents through structure-based virtual screening. J Med Chem 52:4936–4940
14. Shoichet BK (2004) Virtual screening of chemical libraries. Nature 432:862–865
15. Leach AR, Gillet VJ, Lewis RA, Taylor R (2010) Three-dimensional pharmacophore methods in drug discovery. J Med Chem 53:539–558
16. Steindl T, Langer T (2004) Influenza virus neuraminidase inhibitors: generation and comparison of structure-based and common feature pharmacophore hypotheses and their application in virtual screening. J Chem Inf Comput Sci 44:1849–1856
17. Disocovery studio 2.5 software package AI, San Diego, CA (USA): http://www.accelrys.com
18. La Motta C, Sartini S, Tuccinardi T, Nerini E, Da Settimo F, Martinelli A (2009) Computational studies of epidermal growth factor receptor: docking reliability, three-dimensional quantitative structure-activity relationship analysis, and virtual screening studies. J Med Chem 52:964–975
19. Rao SN, Head MS, Kulkarni A, LaLonde JM (2007) Validation studies of the site-directed docking program LibDock. J Chem Inf Model 47:2159–2171
20. Jones G, Willett P, Glen RC, Leach AR, Taylor R (1997) Development and validation of a genetic algorithm for flexible docking. J Mol Biol 267:727–748
21. Morris GM, Goodsell DS, Halliday RS, Huey R, Hart WE, Belew RK, Olson AJ (1998) Automated docking using a lamarckian genetic algorithm and empirical binding free energy function. J Comput Chem 19:1639–1662
22. Lineweaver H, Burk D (1934) The determination of enzyme dissociation constants. J Am Chem Soc 56:658–666
23. Burk D (1984) Enzyme kinetic constants: the double reciprocal plot. Trends Biochem Sci 9:202–204
24. Greenidge PA, Carlsson B, Bladh LG, Gillner M (1998) Pharmacophores incorporating numerous excluded volumes defined by X-ray crystallographic structure in three-dimensional database searching: application to the thyroid hormone receptor. J Med Chem 41:2503–2512
25. Norinder U (2000) Refinement of catalyst hypotheses using simplex optimisation. J Comput Aided Mol Des 14:545–557
26. Palomer A, Cabre F, Pascual J, Campos J, Trujillo MA, Entrena A, Gallo MA, Garcia L, Mauleon D, Espinosa A (2002) Identification of novel cyclooxygenase-2 selective inhibitors using pharmacophore models. J Med Chem 45:1402–1411
27. Shaw DE (2005) A fast, scalable method for the parallel evaluation of distance-limited pairwise particle interactions. J Comput Chem 26:1318–1328
28. Bowers KJ, Chow E, Xu H, Dror RO, Eastwood MP, Gregersen BA, Klepeis JL, Kolossváry I, Moraes MA, Sacerdoti JK, Shan Y, Shaw DE (2006) Scalable algorithms for molecular dynamics simulations on commodity clusters. In: Proceedings of the ACM/IEEE conference on supercomputing (SC06), Tampa, Florida, November 11–17
29. Bowers KJ, Dror RO, Shaw DE (2006) The midpoint method for parallelization of particle simulations. J Chem Phys 124:184109
30. Bowers KJ, Dror RO, Shaw DE (2007) Zonal methods for the parallel execution of range-limited N-body simulations. J Comput Phys 221:303–329
31. Chillemi G, D'Annessa I, Fiorani P, Losasso C, Benedetti P, Desideri A (2008) Thr729 in human topoisomerase I modulates anti-cancer drug resistance by altering protein domain communications as suggested by molecular dynamics simulations. Nucleic Acids Res 36:5645–5651
32. Marco E, Laine W, Tardy C, Lansiaux A, Iwao M, Ishibashi F, Bailly C, Gago F (2005) Molecular determinants of topoisomerase I poisoning by lamellarins: comparison with camptothecin and structure-activity relationships. J Med Chem 48:3796–3807
33. Kennedy WP, Kaminski JM, Van der Ven HH, Jeyendran RS, Reid DS, Blackwell J, Bielfeld P, Zaneveld LJ (1989) A simple, clinical assay to evaluate the acrosin activity of human spermatozoa. J Androl 10:221–231
34. Hortin GL, Warshawsky I, Laude-Sharp M (2001) Macromolecular chromogenic substrates for measuring proteinase activity. Clin Chem 47:215–222
35. Yang TC, Liu YR (1994) Kinetics of inhibitory effects of *Phaseolus calcaratus* inhibitor on acrosin of human body. J Fujian Normal University (Natural Science) 10:8–12