Chem Biol Drug Des 2013; **81:** 437–441

Research Letter

Fragment-Based Design of Novel Quinazolinon Derivatives as Human Acrosin Inhibitors

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Human acrosin is a promising target for the male contraceptives. On the basis of the active site of human acrosin, a series of novel quinazolinon compounds were designed by a fragment docking and growing strategy. *In vitro* anti-acrosin assay revealed that all the compounds showed potent human acrosin inhibitory activities. In particular, compounds 5c and 5g are more active than the known inhibitors. Molecular docking studies revealed that the quinazolinon inhibitors interacted with human acrosin mainly through hydrogen bonding and hydrophobic interactions. The binding mode was also consistent with the structure–activity relationships. The quinazolinon derivatives in this study can serve as new lead structure for the development of novel male contraceptives.

Key words: binding mode, human acrosin, lead structure, male contraceptives, quinazolinon inhibitor

Abbreviations: AIBN, azodiisobutyronitrile; FBDD, fragment-based drug design; MCSS, multiple copy simultaneous search; NBS, *N*-bromosuccinimide.

Received 15 October 2012, revised 2 January 2013 and accepted for publication 10 January 2013

Fertility control is an important and global issue because of overpopulation and unintended pregnancy. In contrast to various hormonal-based oral contraceptives for women, the discovery and development of effective, safe, and reversible male contraceptive remain a challenge. Human acrosin is a serine protease that is located in epididymis and plays an important role during the process of fertilization (1). The functions of human acrosin during fertilization include acrosomal exocytosis, the receptor for zona pellucida proteins, and facilitating the penetration of spermatozoa into the egg (2). Because of its special location and high cell specificity, human acrosin has been regarded as a potential target for the development of novel male contraceptive agents. Currently, known human acrosin inhibitors include bdellins (3), *N*-alpha-tosyl-L-lysyl-chloromethylketone (TLCK) (4), suramin (5), *p*-aminobenzamidine (pAB) (6), isoxazolecarbaldehydes (7), and 4-guanidinobenzoates (8) (Figure 1). However, most of them have low affinity, poor selectivity, and high toxicity. In this scenario, rational inhibitor design on the basis of the three-dimensional (3D) structure of human acrosin is an effective way to find novel lead structures.

In our previous studies, 3D model of human β-acrosin has been constructed by homology modeling (9) using the crystal structures of both ram and boar acrosin (10) as the templates. The active site of human acrosin and the binding mode of known inhibitors were investigated by the multiple copy simultaneous search (11) and molecular docking (12). Moreover, novel human acrosin inhibitors have been identified by virtual screening (13). In recent years, fragment-based drug design (FBDD) has been emerged as a promising approach for lead discovery and optimization (14,15). Herein, we describe fragment-based rational design and synthesis of new quinazoline ketone derivatives as potent human acrosin inhibitors.

The first step of structure-based drug design is to analyze the properties of the active site. As shown in Figure 2A, the active site of human acrosin can be divided into three parts: P1, P2, and G pocket. The P1 pocket is a polar and deep site lined with residues Thr216, Cys217, Gln218, Trp243, and Gly244, which are important for inhibitor binding. The entrance of the P2 pocket is larger than the P1 pocket, and important residues in this area include Arg199, Tyr196, Gly244, Val255, and Val245. The G pocket is a narrow channel that is surrounded by Glu120, His69, Trp243, and Ala68. In particular, Trp243 is located near the center of the P1, P2, and G pocket and can be used as the starting point for drug design. Thus, the following task is to find a suitable chemical scaffold that can be located in the center of the active site and interact with Trp243. The scaffold library was obtained from Ji's *de novo* design method fragment hopping (16). The fragment-like molecules were docked into the active site of human acrosin using AUTODOCK (17). The fragments were ranked by the binding energy, and the top 10 scored fragments (see Appendix S1 in Supporting information) were subjected for visual inspection of their binding mode.

 doi: 10.1111/cbdd.12106

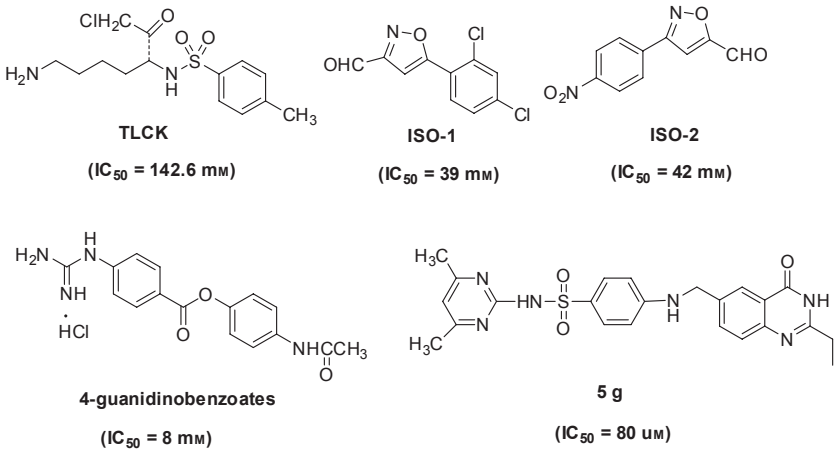
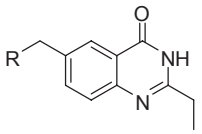


Figure 1: Chemical structures of human acrosin inhibitors and compound **5g**.

Table 1: Chemical structure, human acrosin inhibitory activity (IC_{50}, mM), and binding energy (Kcal/mol) of the quinazolinon derivatives



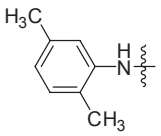
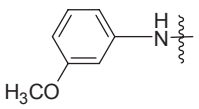
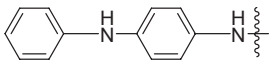
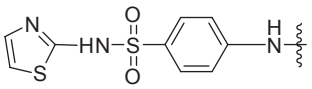
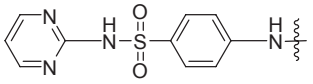
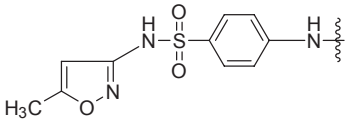
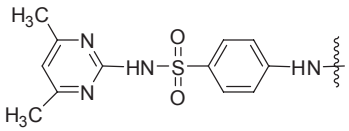
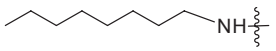
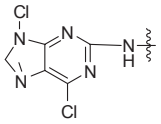
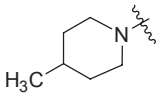
Compounds	R	IC_{50}	Binding energy
3	H	5.3	−7.80
5a		0.98	−9.83
5b		0.84	−9.61
5c		0.25	−11.31
5d		0.78	−10.7
5e		1.3	−8.26
5f		0.82	−9.33

Table 1: continued

Compounds	R	IC_{50}	Binding energy
5g		0.080	−16.24
5h		1.8	−10.4
5i		2.3	−9.98
5j		2.4	−8.37
TLCK		142.6	−8.26

TLCK, *N*-alpha-tosyl-L-lysyl-chloromethylketone.

As a result, quinazolinon was finally selected as the core structure to interact with human acrosin. Molecular docking results revealed that the quinazolinon scaffold **3** was positioned near Trp243 and formed two hydrogen bonds with Trp243 and Gln218, respectively (Figure 2B). To validate the reliability of fragment docking, the acrosin inhibitory activity of the quinazolinon scaffold **3** was determined. To our delight, fragment **3** showed moderate inhibitory activity with an IC_{50} value of 5.3 mM. Then, fragment-based growing strategy was used to improve the binding affinity of the quinazolinon core. In consideration of synthetic accessibility, various amine-containing side chains, such as substituted anilines, aliphatic amines, and heterocycle-sulfonamide-phenyl amines, were designed to be lined to the quinazolinon framework to form additional hydrophobic and hydrogen bonding interactions with the P2 and G pocket. As a result, compounds **5a–j** (Table 1) were subjected to chemical synthesis.

Scheme 1 outlines the synthetic route of the target compounds. Starting from 2-amino-5-methylbenzoic acid (**1**), it was condensed with propionic anhydride to afford benzoxazine-4-one compound **2**. After dehydration with formamide, intermediate 2 was converted to quinazoline derivative **3**. The methyl group of compound **3** was brominated by *N*-bromosuccinimide (NBS) and azodiisobutyronitrile to give bromomethyl derivative **4**. Target compounds **5a–j** were obtained by reaction **4** with various amines in the presence of triethylamine (TEA) and DMF.

The *in vitro* human acrosin inhibitory activities of the synthesized compounds were determined according to the method of Kennedy *et al.* (18). *N*-alpha-tosyl-L-lysyl-chloromethylketone was used as a standard reference drug. The IC_{50} values and AUTODOCK docking energies are listed in Table 1. In general, all the compounds are active against human acrosin with their IC_{50} values in the range of 80 μM to 2.4 mM. In comparison with the activity of known human acrosin inhibitors (Figure 1), significant improvement of the inhibitory activity was observed for the quinazolinon derivatives reported here. Particularly, compound **5g** showed the best inhibitory activity (IC_{50} = 80 μM), which was far more potent than the reference drug TLCK. AutoDock was used to investigate the binding mode of the synthesized compounds. Figure 2B depicts the interaction between representative compound **5g** and the active site of human acrosin. After the attachment of the amine side chain to the scaffold, the position and key interactions of the quinazolinon core are retained. The imine nitrogen atom and amide NH of the quinazolinon ring formed two hydrogen bonds with Trp243 and Gln218, respectively. The amine side chain of compound **5g** was positioned into the G and P2 pocket. The NH linker also formed hydrogen bonding interaction with Glu120 in the G pocket. The sulfonamide group does not form direct interaction with human acrosin. The terminal dimethylpyridine was located in the P2 pocket and formed *van der Waals* and hydrophobic interaction. In addition, the nitrogen atom of the pyridine ring also formed a hydrogen bond with Arg199, which is an important residue to achieve specificity (10).

The binding mode of compound **5g** is supported by the SAR results of the synthesized compounds. Substituted aniline derivatives **5a** and **5b** are more potent

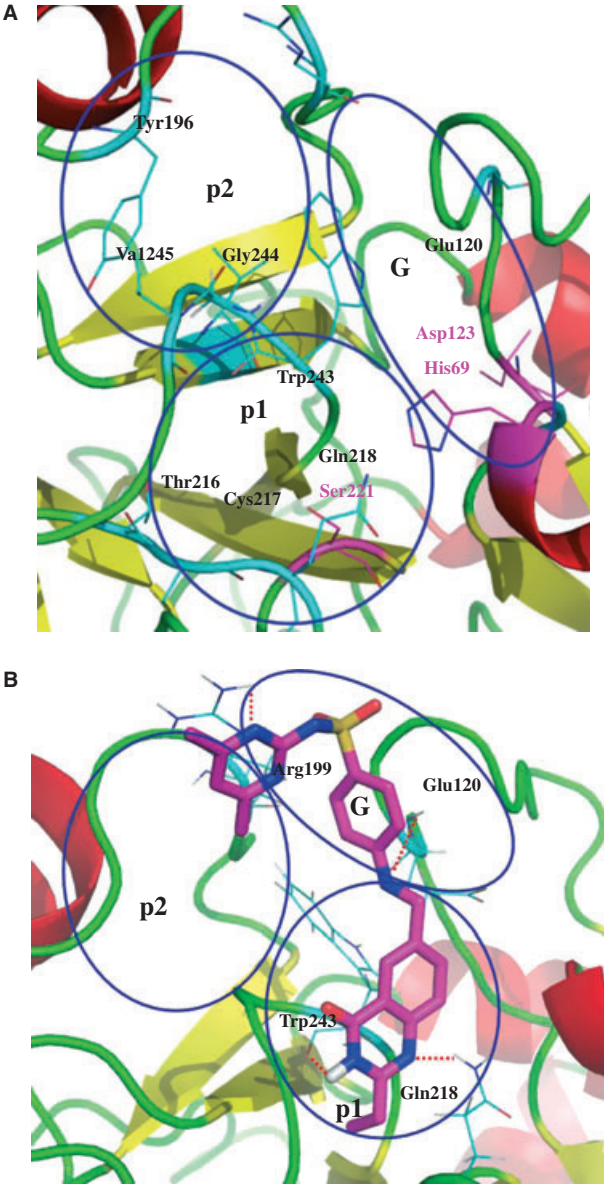


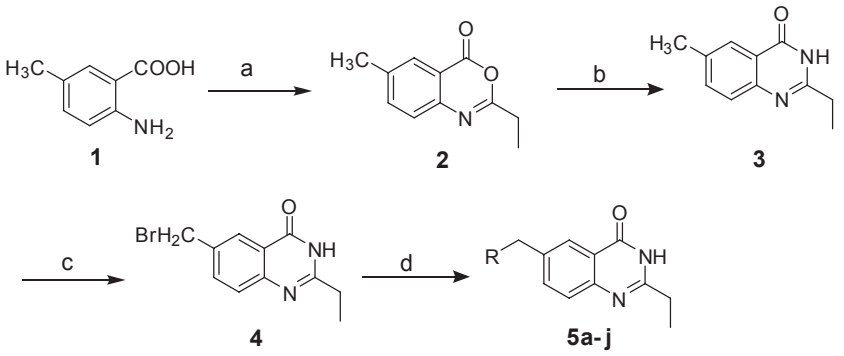
Figure 2: (A) The active site of human acrosin; (B) the binding mode of inhibitor **5g** in the active site of human acrosin and hydrogen bonds are displayed as dotted lines.

than the purine derivative **5i** and piperidine derivative **5j** because they form stronger hydrophobic interaction with the G pocket. However, all of them have short side chain and cannot interact with the P2 pocket. Therefore, they are less active than the compounds with long side chain (e.g., compounds **5c**–**g**), which have lower interaction energies with human acrosin (Table 1). In comparison with compound **5g**, the inhibitory activity of demethyl derivative **5e** is decreased because it forms lower hydrophobic interaction with the P2 pocket. Similarly, the replacement of terminal phenyl group of compound **5c** with less hydrophobic thiazole (compound **5d**) and methylisoxazole (compound **5f**) leads to the decrease in the inhibitory activity. Compound **5g** is the most active compound because its terminal dimethylpyrimidine group can form both strong hydrophobic interaction and additional hydrogen bonding interaction with the P2 pocket. Moreover, several acrosin inhibitors, such as TLCK and isoxazole aldehydes, could bind covalently with acrosin. Because covalent inhibitors are being used more frequently in the pharmaceutical industry, it might be applied to further optimize compound **5g** by incorporating a proper substituent to covalently interact with key residues of acrosin (e.g., Asp189, Ser221).

In summary, computational fragment-based approaches were used to design a series of quinazolinon derivatives as novel human acrosin inhibitors. The quinazolinon derivative showed improved inhibitory activity over the known human acrosin inhibitors. The most active compound **5a** has good drug-like properties, which represents a good starting point for lead optimization. Furthermore, the fragment docking and fragment growing strategy used in this study should have general implications for structure-based rational drug design.

Acknowledgment

This work was supported by the Shanghai population and Family Planning Commission (Grant Nos. 2007JG-02) and National Natural Science Foundation of China (Grant No. 81222044).



Scheme 1: Reagents and conditions: (a) Propionic anhydride, reflux, 5 h, 69.3%; (b) $HCONH_2$, 150 °C, 7 h, 75.5%; (c) *N*-bromosuccinimide (NBS), azodiisobutyronitrile (AIBN), $CHCl_3$, reflux, 4 h, 67.8%; (d) substituted amines, Et_3N, DMF, 10 h, 6.9–98.7%.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Top 10 ranked scaffolds for fragment-based drug design. Experimental details, NMR and MS data for the synthesized compounds. The binding mode of scaffold **3** in the active site of human acrosin. Molecular weight (MW), ligand efficiency (LE) and ligand-lipophilicity efficiency of the target compounds.