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Design and Synthesis of Phenylisoxazole Derivatives as Novel Human Acrosin Inhibitors

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Abstract

Human acrosin is an attractive target for the discovery of novel male contraceptives. Isoxazole derivative ISO-1, a small-molecule weak human acrosin inhibitor, was used as the starting point for lead optimization. After two rounds of structure-based inhibitor design, a highly potent inhibitor **B6** (IC_{50} = 1.44 μM) was successfully identified, which showed good selectivity over trypsin and represents one of the most active human acrosin inhibitors up to date.

Keywords: Guanidinophenylpyrazole derivatives, Molecular hybridization, Male contraceptive, Human acrosin inhibitors

It is estimated that human population in the world will reach about 9 billion in the year 2050 [1]. Overpopulation is becoming one of the major global issues because of limited resources and increased environmental burdens [1]. Contraception is one of the best solutions to solve these problems. Currently, most of the contraceptives are developed for women. However, choices for male contraception are rather limited (*e.g.* condom and vasectomy). Discovery and development of effective male contraceptives represents a challenging research area [2].

Acrosin is a typical serine endoprotease with trypsin-like activity, which is stored in its inactive zymogen form (called proacrosin) and secreted as a serine protease after sperm–zona pellucida (ZP) binding [3]. Acrosin plays multiple roles during fertilization in several significant ways including activation of acrosome components, secondary binding with ZP, and hydrolysis of ZP. Due to the importance of human acrosin in fertilization, it is an attractive target for the development of human male contraceptives. In 2000, crystal structures of ram and boar β-acrosins in complex with *p*-aminobenzamidine were solved, which provided structural basis for rational design of acrosin inhibitors [4].

Up to now, small molecule human acrosin inhibitors mainly included TLCK [5], DV-1006, *p*-aminobenzamidine (pAB) [6], and isoxazolecarbaldehydes (ISO-1) [7] (**Fig. 1**). However, most of these inhibitors showed relatively poor human acrosin inhibitory activity (IC_{50} values in mM range). Structure-based drug design represents an effetive stragegy to disocver potent human acorsin inhibitors. However, three dimensional structure of human acrosin has not been solved up to date. Thus, we

contructed a structural model of human acrosin using homology modeling [8]. Moreover, three important sub-sites (P1, P2 and G) for inhibitor binding were identified by computational analysis of the active site of human acrosin [9]. Based on information obtained from computer modeling, we successfully designed a number of novel acrosin inhibitors including 4-guanidinobenzoates [8], diaminomethylene-benzenesulfonamides [10], benzenesulfonamides [11], phenylpyrazoles [12], imidazolesulfonamides [13], phenylpyrazoles [14] and quinazolinons [15] (**Fig. 1**).

Continuing our efforts in the discovery of novel acrosin inhibitors, herein the isoxazolecarbaldehyde inhibitor **ISO-1** was used as a starting point for lead optimization. **ISO-1** is a fragment-like lead compound with a low molecular weight (MW = 240). Although its activity was low, structure-based methods can be used to improve its potency. A series of phenylisoxazole derivatives were designed and synthesized and most of them showed significantly increased human acrosin inhibitory activity. Particularly, a highly potent inhibitor (compound **B6**) was identified and its binding mode was clarified by molecular docking.

Chemical synthesis of phenylisoxazole derivatives **A1**-**A5** is outlined in **Scheme 1**. 1-(4-Nitrophenyl)ethanone (**1**) was oxalylated by diethyl oxalate in the presence of sodium ethanol to afford diketoester **2**. The product was condensed with hydroxylamine hydrochloride to yield the isoxazole scaffold **3**. Then, the nitro group of intermediate **3** was reduced by $SnCl_2$ and followed by acylation with various acyl chlorides in the presence of triethylamine to give the target compounds **A1**-**A5**. Target

compounds **B1**-**B25** were synthesized by the method outlined in **Scheme 2**. The guanidino intermediate **5** was prepared by reacting the amino group of intermediate **4** with NH_2CN using EtOH as the solvent and HCl as the acid. Catalyzed by NaOH, the ester group of compound **5** was hydrolyzed and finally condensed with various amines in the presence of 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC·HCl) and *N*-hydroxybenzotriazole (HOBt) to give the target compounds **B1**-**B25**.

Lead optimization of ISO-1 was based on three subsites (P1, P2 and G) in the active site of human acrosin [9]. Docking studies revealed that ISO-1 was located in the P1 pocket (a hydrophobic hydrogen-bonding site) with its aldehyde oxygen atom forming a hydrogen bond with Thr216 (**Fig. 2A**). On the basis of the binding model, a series of phenylisoxazole derivatives (**A1**-**A5**) were designed. First, the phenylisoxazole scaffold of **ISO-1** was extended into the P2 pocket to form additional interactions by introducing a substituted phenyl amide group. Second, the chemically unstable aldehyde group was replaced by an ester group, which was expected to form hydrogen bonding interaction with Gln218.

In vitro human acrosin inhibitory assay was performed using the protocols according to our previous study [11]. The results revealed that most of the target compounds were more active than the lead compound **ISO-1** with IC_{50} values in the range of 60 μM to 460 μM (**Table 1**). Particularly, the 2-chlorine derivative **A2** showed the best activity (IC_{50} = 60 μM), which was 6 fold more potent than **ISO-1**. Compound **A2** was docked into the active site of human acrosin using LibDock

within Discovery Studio 2.5 software package (DS 2.5) [16]. As shown in **Fig. 2B**, the isoxazole scaffold was located into the P1 pocket with its nitrogen atom and oxygen atom forming two hydrogen bonds with Ser221 and Gln218, respectively. As expected, the carbonyl oxygen atom of the ester group also formed a hydrogen bond with the backbone amide of Gln218. The 2-chlorophyenyl amide group was extended into the P2 pocket and formed hydrophobic contacts.

The guanidyl group was an important pharmacophore observed in several human acrosin inhibitors (*e.g.* KF950), because it could form hydrogen bonding interactions with the P1 pocket [9]. Therefore, the guanidyl group was introduced onto the phenylisoxazole inhibitors to increase the inhibitory potency. A series of guanidinophenylisoxazole derivatives (**B1**-**B25**) were designed and synthesized. To our delight, most of the target compounds showed significantly improved human acrosin inhibitory activities (**Table 2**). Notably, nine highly active compounds (*i.e.* **B5**, **B6**, **B7**, **B8**, **B10**, **B15**, **B18**, **B19** and **B22**) were successfully identified with IC_{50} values lower than 10 μM. In particular, compound **B6** showed the best activity (IC_{50} = 1.4 μM), which was far more potent than the lead compound **ISO-1**. In order to investigate the selectivity of compound **B6**, we determined its inhibitory activity against pig trypsin (a member of the serine protease family). The IC_{50} value was 4200 μM, indicating that compound **B6** exhibited good specificity toward human acrosin.

Structure-activity relationship (SAR) analysis indicated that the guanidine group was essential for acrosin inhibitory activity. Moreover, the phenyl substitution attached to the amine group was also important because the alkyl derivatives **B1** and

B2 were inactive. The type and position of the substitutions on the terminal phenyl group had various effects on the inhibitory activities. As compared with the unsubstituted phenyl derivative **B3** (IC_{50} = 24.6 μM), the introduction of various substitutions generally led to the increase of the activity. In particular, compounds with di-methoxyl substitutions, namely **B5** and **B6**, showed excellent inhibitory activities at low micromolar concentrations. The 2,5-dimethoxyl derivative **B6** (IC_{50} = 1.4 μM) was the most active compound in this series. In contrast, mono-methoxyl derivatives **B18** (IC_{50} = 8.4 μM) and **B9** (IC_{50} = 6.6 μM) showed decreased activity. Moreover, di-methyl derivatives (*e.g.* **B10**, **B12** and **B24**) were also less active than the di-methoxyl derivatives. When the phenyl group was enlarged to naphthalene (**B21**, IC_{50} = 29.9 μM), the activity was largely decreased. Moreover, ligand efficiency (LE) values of the guanidinophenylisoxazole derivatives were calculated (Table 2). Most of the compounds had similar LE values ranging from 0.22 to 0.28. Compound B6 showed the best LE value (0.28) and o deserved to be further optimized.

Molecular docking studies revealed that the general conformation of compound **B6** was different from that of the phenylisoxazole inhibitor **A2** (**Fig. 2C**). It mainly interacted with the P1 and G pocket, whose conformation was directed by the hydrogen bonding interaction between the guanidyl group and acrosin. As expected, the guanidyl group was located deep into the P1 pocket and formed five hydrogen bonds with Tyr256, Trp243, Thr216, and Val225, respectively. Moreover, cation-π interaction was also observed between the guanidyl group and Tyr256 (**Fig. 3**). The

dimethoxylphenyl group formed hydrophobic interactions with Val245 and cation-π interaction with Arg248.

In summary, structure-based drug design was successfully applied to optimize the isoxazole human acrosin inhibitor **ISO-1**. Most of the target compounds showed significantly improved human acrosin inhibitory activities. After two round of inhibitor design, a highly potent guanidinophenylisoxazole inhibitor **B6** (IC_{50} = 1.44 μM) with good selectivity toward trypsin was identified. Molecular docking studies revealed that it formed hydrogen bonding, hydrophobic and cation-π interactions with the active site of human acrosin. Moreover, compound **B6** has suitable physicochemical properties [17], showing its potential as an orally active agent. Thus, compound **B6** represented a promising lead to develop novel male contraceptives. Further structural optimization studies are in progress.

Supplementary Material

Experimental Protocols and Structural Characterization of Target Compounds.

Acknowledgments

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References and Notes

[1] J. Cleland, S. Bernstein, A. Ezeh, A. Faundes, A. Glasier, J. Innis, Family planning: the unfinished agenda, Lancet, 368 (2006) 1810-1827.

[2] K.W. Mok, D.D. Mruk, P.P. Lie, W.Y. Lui, C.Y. Cheng, Adjudin, a potential male

contraceptive, exerts its effects locally in the seminiferous epithelium of mammalian testes, Reproduction, 141 (2011) 571-580.

[3] H.T. Mao, W.X. Yang, Modes of acrosin functioning during fertilization, Gene, 526 (2013) 75-79.

[4] R. Tranter, J.A. Read, R. Jones, R.L. Brady, Effector sites in the three-dimensional structure of mammalian sperm beta-acrosin, Structure, 8 (2000) 1179-1188.

[5] R. Pakzad, [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 (1989) 8-13.

[6] L.R. Fraser, p-Aminobenzamidine, an acrosin inhibitor, inhibits mouse sperm penetration of the zona pellucida but not the acrosome reaction, J Reprod Fertil, 65 (1982) 185-194.

[7] G. Gupta, R.K. Jain, J.P. Maikhuri, P.K. Shukla, M. Kumar, A.K. Roy, A. Patra, V. Singh, S. Batra, Discovery of substituted isoxazolecarbaldehydes as potent spermicides, acrosin inhibitors and mild anti-fungal agents, Hum Reprod, 20 (2005) 2301-2308.

[8] J.G. Lv, C.Q. Sheng, M. Zhang, H.T. Ji, W.N. Zhang, Y.J. Zhou, J. Zhu, J.H. Jiang, Homology Modeling of Human Acrosin and Its Molecular Docking Study with KF950, Acta Chim Sinica, 64 (2006) 1073-1078.

[9] J. Zhang, C.H. Zheng, C.Q. Sheng, Y.J. Zhou, J. Zhu, J.G. Lv, Characterization of Human Acrosin Active Site and Binding Modes with Its Inhibitors, Chem J Chinese U, 30 (2009) 2409-2414.

[10] L. Ding, J. Zhu, C. Zheng, C. Sheng, J. Qi, X. Liu, G. Han, J. Zhao, J. Lv, Y. Zhou, Synthesis and acrosin inhibitory activity of substituted 4-amino-N-(diaminomethylene) benzenesulfonamide derivatives, Bioorg Med Chem Lett, 21 (2011) 6674-6677.

[11] X. Liu, G. Dong, J. Zhang, J. Qi, C. Zheng, Y. Zhou, J. Zhu, C. Sheng, J. Lu, Discovery of novel human acrosin inhibitors by virtual screening, J Comput Aided Mol Des, 25 (2011) 977-985.

[12] J. Qi, J. Zhu, X. Liu, L. Ding, C. Zheng, G. Han, J. Lv, Y. Zhou, Synthesis and acrosin inhibitory activities of substituted ethyl 5-(4-aminophenyl)-1H-pyrazole-3-carboxylate derivatives, Bioorg Med Chem Lett, 21 (2011) 5822-5825.

[13] Q. Chen, W. Tian, G. Han, J. Qi, C. Zheng, Y. Zhou, L. Ding, J. Zhao, J. Zhu, J. Lv, C. Sheng, Design and synthesis of novel benzoheterocyclic derivatives as human acrosin inhibitors by scaffold hopping, Eur J Med Chem, 59 (2013) 176-182.

[14] W. Tian, G. Han, J. Zhu, J. Qi, Q. Chen, J. Zhao, C. Zheng, L. Zhang, Y. Zhou, J. Lv, Synthesis and acrosin inhibitory activities of 5-phenyl-1H-pyrazole-3-carboxylic acid amide derivatives, Bioorg Med Chem Lett, 23 (2013) 4177-4184.

[15] W. Ning, J. Zhu, C. Zheng, X. Liu, Y. Song, Y. Zhou, X. Zhang, L. Zhang, C. Sheng, J. Lv, Fragment-based design of novel quinazolinon derivatives as human acrosin inhibitors, Chem Biol Drug Des, 81 (2013) 437-441.

[16] S.D. Disocovery studio 2.5 software package AI, CA (USA): http://www.accelrys.com.

[17] Calculated physicochemical properties of compound B6: molecular weight = 381, number of hydrogen bond donors = 4, number of hydrogen bond donors = 9, LogP = 2.03, tPSA = 131.05.

Figure Legends

Figure 1. Chemical structures of known human acrosin inhibitors.

Figure 2. The binding modes of compounds **ISO-1** (**A**), **A2** (**B**), and **B6** (**C**) with the active site of human acrosin.

Figure 3. Schematic representation of the proposed interactions between inhibitor B6 and the active site of human acrosin.

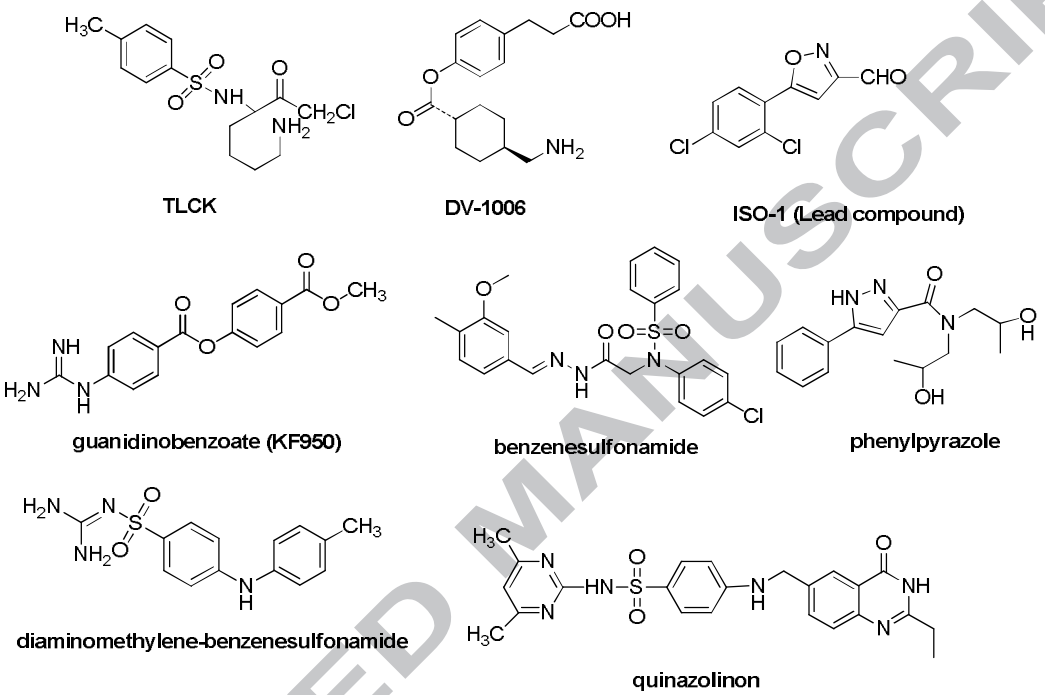
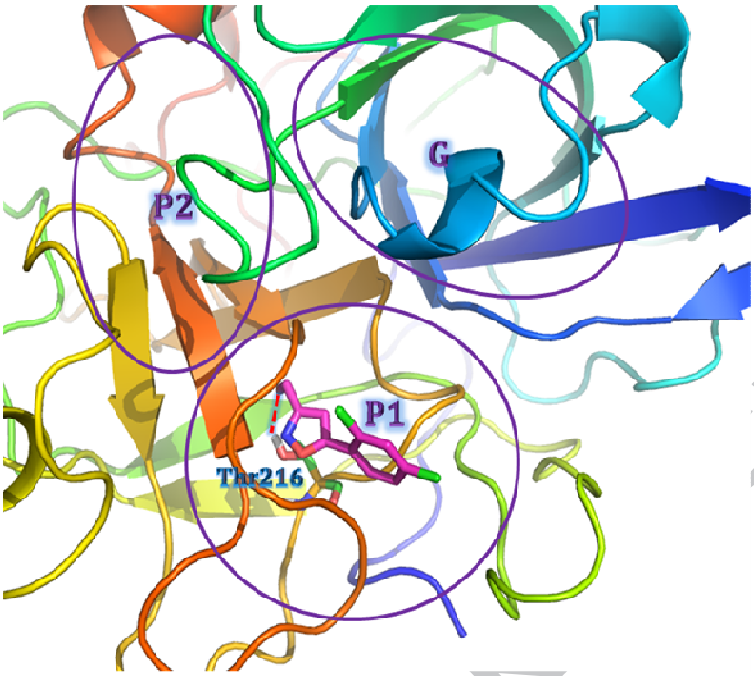
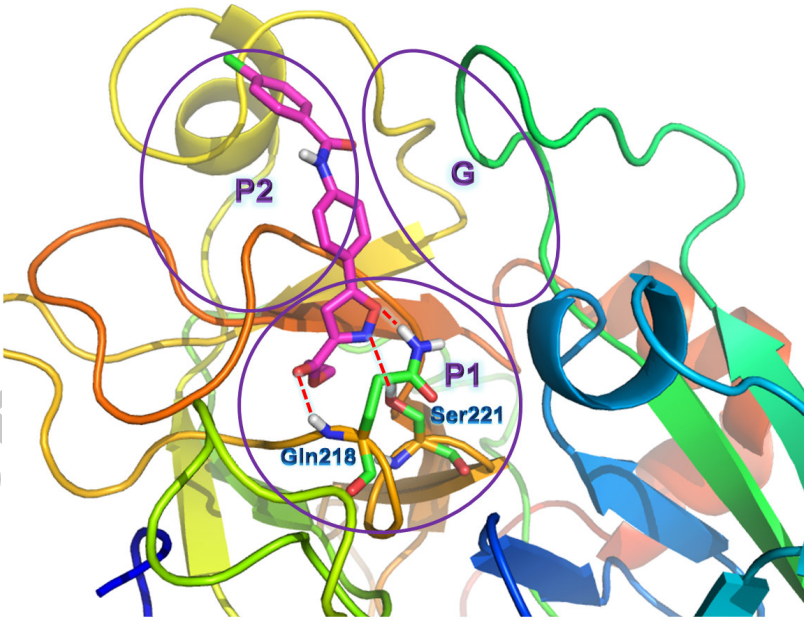


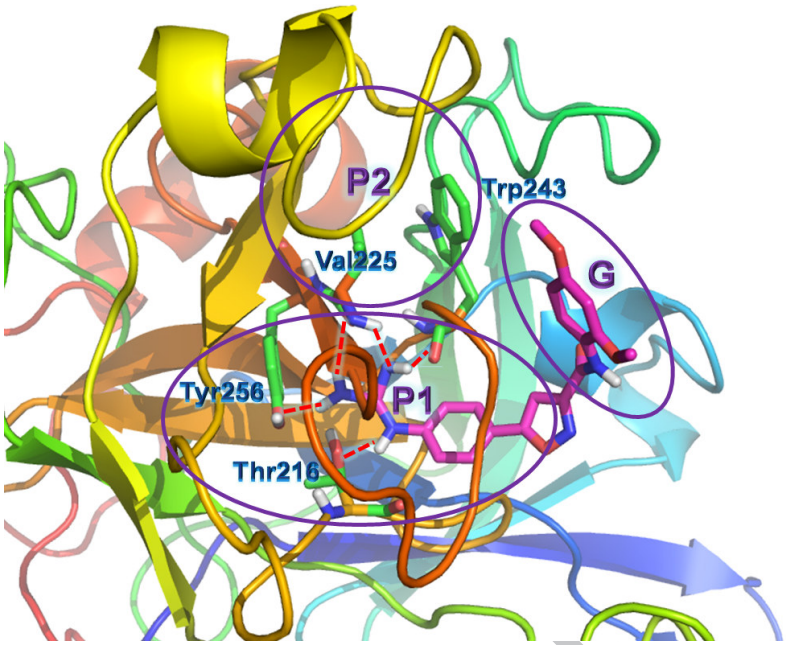
Figure 1. Chemical structures of known human acrosin inhibitors.



(A)



(B)



(C)

Figure 2. The binding modes of compounds **ISO-1** (**A**), **A2** (**B**), and **B6** (**C**) with the active site of human acrosin.

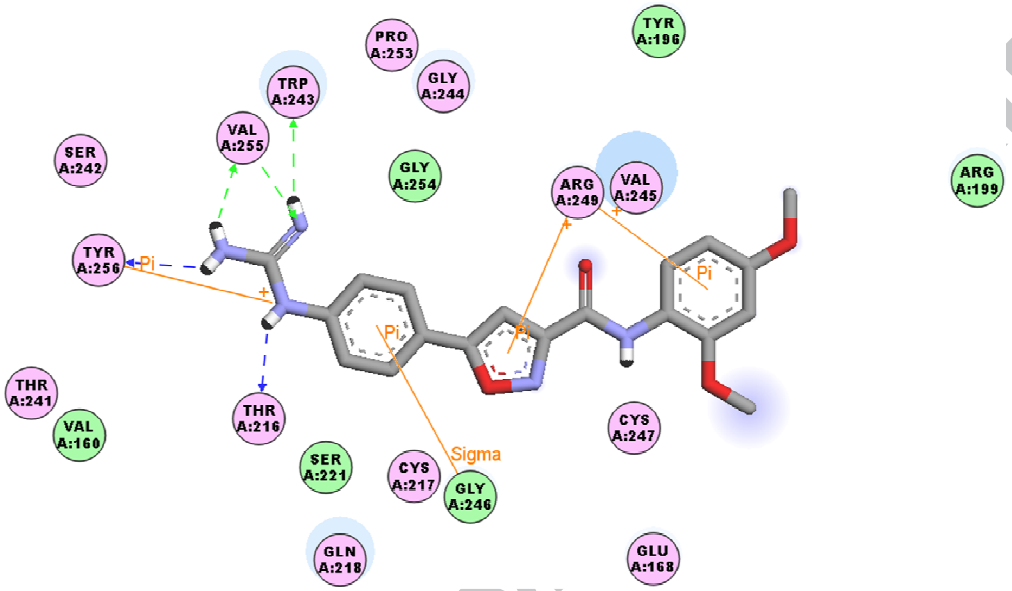
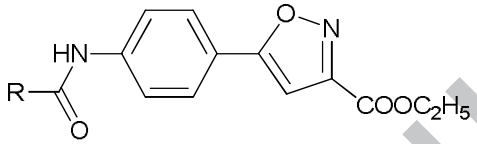


Figure 3. Schematic representation of the proposed interactions between inhibitor B6 and the active site of human acrosin.

Table 1. Chemical structures and human acrosin inhibitory activities of the phenylisoxazole derivatives



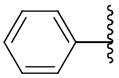
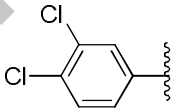
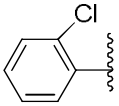
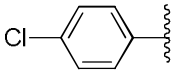
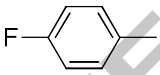
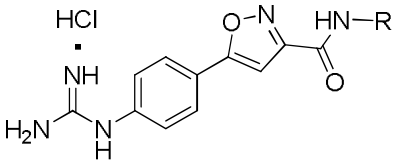
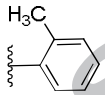
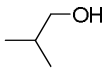
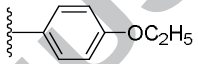
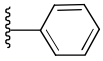
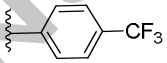
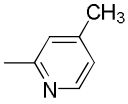
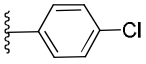
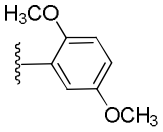
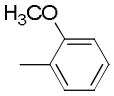
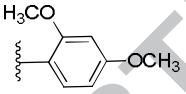
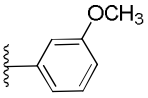
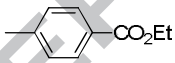
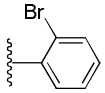
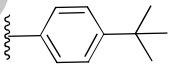
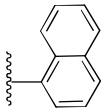
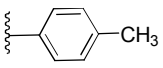
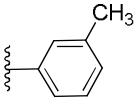
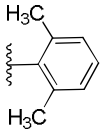
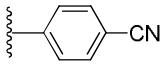
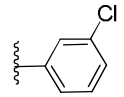
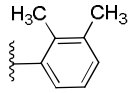
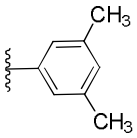
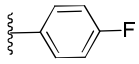
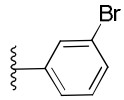
Compds	R	IC_{50} (μM)	Compds	R	IC_{50} (μM)
A1		460.0	**A4**		240.0
A2		60.0	**A5**		380.0
A3		140.0	**ISO-1**		390.0

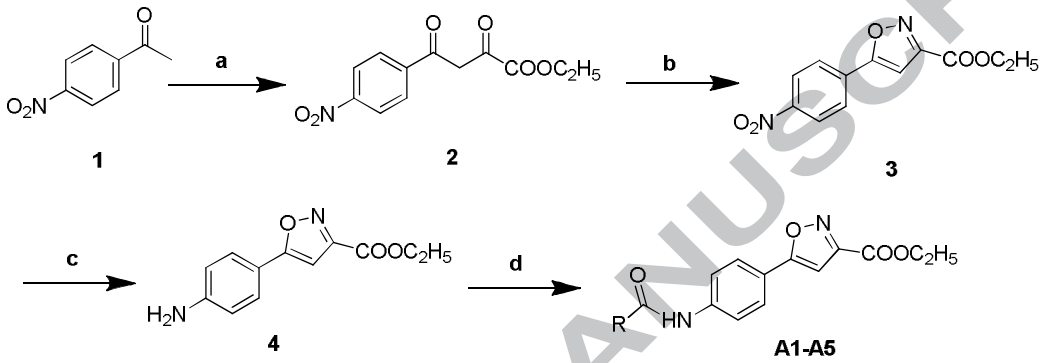
Table 2. Chemical structures, human acrosin inhibitory activities (IC_{50}, μM) and ligand efficiency of the guanidinophenylisoxazole derivatives



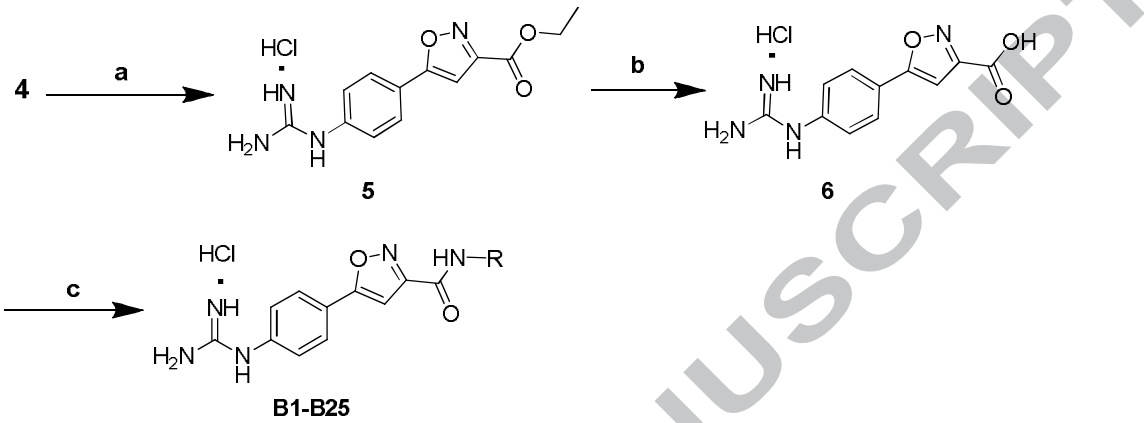
Compds	R	IC_{50}	LE[a]	Compds	R	IC_{50}	LE[a]
B1		>100	0.26	**B14**		14.4	0.26
B2		>100	0.25	**B15**		6.4	0.26
B3		24.6	0.26	**B16**		20.3	0.23
B4		21.4	0.25	**B17**		26.8	0.25
B5		2.3	0.27	**B18**		8.4	0.27
B6		1.4	0.28	**B19**		6.6	0.27
B7		9.3	0.24	**B20**		27.9	0.25
B8		7.8	0.25	**B21**		29.9	0.22
B9		10.1	0.27	**B22**		9.4	0.27
B10		5.7	0.27	**B23**		23.9	0.24

B11		25.4	0.25	**B24**		11.5	0.26
B12		12.1	0.26	**B25**		20.5	0.26
B13		14.4	0.26	**ISO-1**		390.0	

[a] LE = ($-\Delta G$ / HAC $\approx$ $-RT \ln(IC_{50})$ / HAC), HAC = heavy atom count.



Scheme 1. Reagents and conditions: (a) $(COOC_2H_5)_2$, C_2H_5ONa, rt, 24 h, yield 90.6%; (b) $H_2NOH{\cdot}HCl$, EtOH, reflux, 4 h yield 81.9%; (c) $SnCl_2{\cdot}2H_2O$, EtOAc, reflux, 6 h, yield 81.9%; (d) Acyl chlorides, Et_3N, $CH_3CO_2C_2H_5$, yields 42.0%~81.0%.

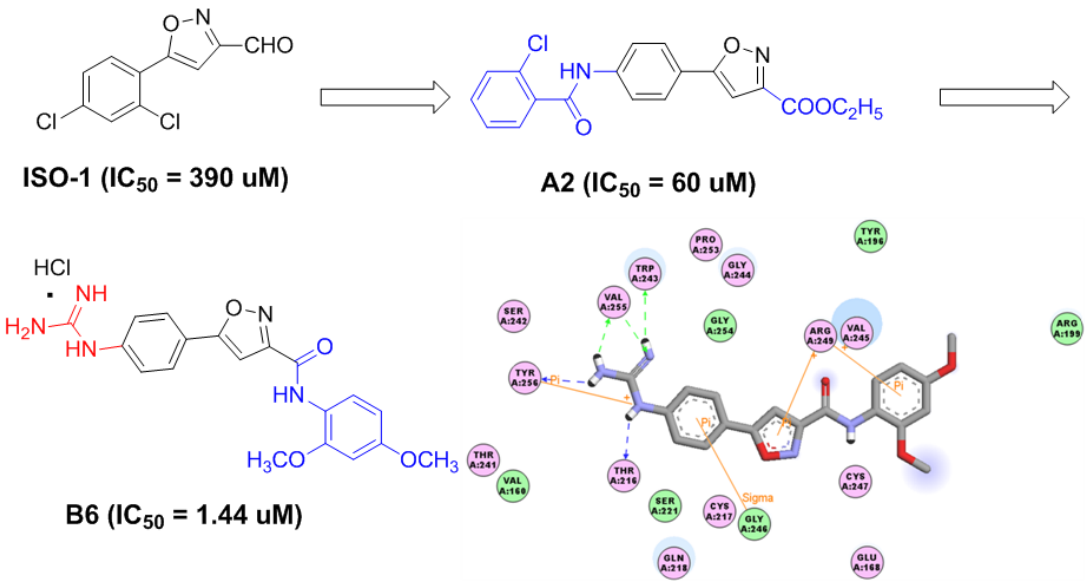


Scheme 2. Reagents and conditions: (a) H_2NCN, HCl, EtOH, reflux, 8h, yield 83.9%; (b) NaOH, CH_3OH/H_2O (1:1), reflux, 2 h, yield 92.2%; (c) ROH or RNH_2, EDC·HCl, HOBt, rt, 48h, yields 43.0%~90.0%

Graphical Abstract

Discovery of Novel Phenylisoxazole Human Acrosin Inhibitors by Structure-based Drug Design

Juntao Zhao[§], Wei Tian[§], Jingjing Qi[§], Diya Lv, Yang Liu, Yan Jiang, Guoqiang Dong, Qianqian Chen, Youjun Zhou, Ju Zhu, Heling Wang, Chunquan Sheng* and Jiaguo Lv*



A potent human acrosin inhibitor **B6** (IC_{50} = 1.44 μM) was identified by two-round of structure-based drug design.