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Short communication

Design and synthesis of novel benzoheterocyclic derivatives as human acrosin inhibitors by scaffold hopping

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ABSTRACT

Human acrosin is an attracting target for the development of novel male contraceptives. Scaffold hopping was used to optimize the isoxazolecarbaldehyde human acrosin inhibitors and extend their structure–activity relationships. Four kinds of scaffolds, namely benzimidazole, benzothiazole, 3*H*-indazole, and 5-phenyl-1*H*-pyrazole, were designed and synthesized. Most of the synthesized compounds showed potent human acrosin inhibitory activity and their binding modes were investigated by molecular docking. The scaffold of the compounds was found to be important for the inhibitory activity. Several compounds were more active than the positive control TLCK, suggesting that they can serve as good starting points for the discovery of novel male contraceptive agents.

1. Introduction

Fertility control is an important and global issue because of overpopulation and unintended pregnancy. The contraceptive pill is one of the most important methods of contraception, protecting millions of couples worldwide from unwanted pregnancy and allowing them to take control over their reproduction. However, the oral contraceptive pill is only available for women and most of these pills have serious side effects, such as breakthrough menstrual bleeding, nausea, vomiting, acne, breast tenderness, cramping, weight gain, heavy bleeding and headaches [1–4]. Meanwhile, condoms do provide dual protection if used correctly and consistently, but they are not women controlled. Therefore, it is necessary to search for new contraceptive agents which are easier to use with fewer side effects and available for both men and women.

Acrosin is a multifunctional protein located in acrosome, which plays a critical role in the reproduction process. It not only affects motility of sperm but also disperses acrosomal matrix and hydrolyzes the zona pellucida, which helps sperm enter and fusion to ovum [5]. Insufficient levels of acrosin are linked with infertility and inhibition of acrosin activity by protease inhibitors has been shown to reduce the success of fertilization [6]. Therefore contraception can be achieved through acrosome enzyme inhibition. In recent years, acrosin has become an attractive target in contraceptive drug discovery. Since the function of acrosin was reported, there have been a large number of research interests focusing on the discovery of novel acrosin inhibitors. A number of small molecule acrosin inhibitors have been reported, such as TLCK ($C_{14}H_{21}ClN_2O_3S$, CAS No. 2104-86-1), AGB ($C_{16}H_{16}N_4O_3$, CAS No. 79126-29-7), DV-1006 ($C_{17}H_{23}NO_4$, CAS No. 34675-84-8), isoxazolecarbaldehydes, and guanidylbenzenesulfonamides (KF950, $C_{16}H_{15}N_3O_4$, CAS No. 99450-778) (Fig. 1) [7–9]. Among them, isoxazolecarbaldehyde presents a promising lead structure for the development of potent spermicidal agents with complementary properties that add to their utility as safe, effective and prophylactic topical contraceptives [9]. Thus, the extension of the structure–activity relationships (SARs) of the isoxazolecarbaldehyde inhibitors is of great importance.

In our previous studies, a homologous three-dimensional (3D) model of human acrosin was built based on the crystal structures of ram and boar acrosins [10]. The active site of human acrosin was explored by multiple copy simultaneous search (MCSS) calculations

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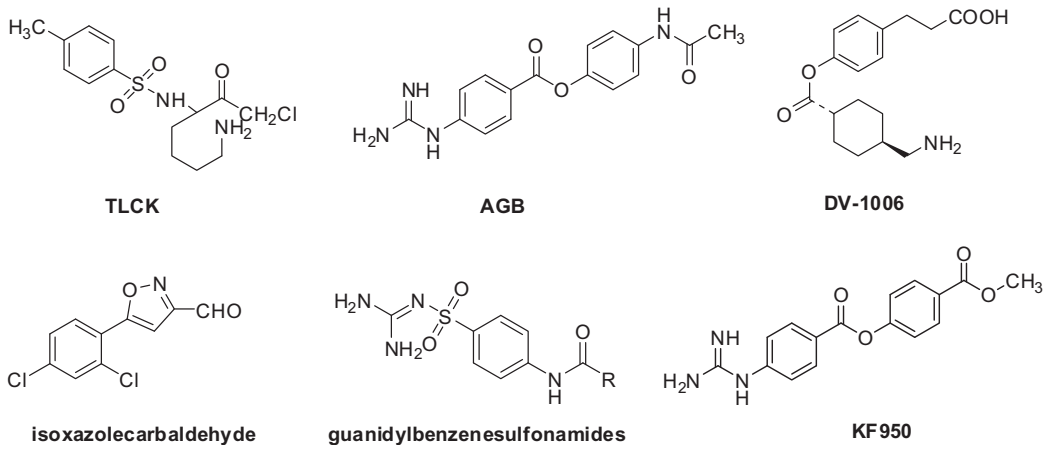


Fig. 1. Chemical structures of representative acrosin inhibitors.

[11]. Guided by the results from molecular modeling, a highly potent acrosin inhibitor $C_{16}H_{15}N_3O_4$ (CAS.99450-778, KF950) was designed and synthesized, which is under pre-clinical trial [10]. Continuing our efforts in rational design of novel acrosin inhibitors [12–14], the isoxazolecarbaldehyde acrosin inhibitor was used as a lead for further structural optimization. Herein, a series of benzoheterocyclic derivatives were designed and synthesized by scaffold hopping (Fig. 2). *In vitro* assay indicated that several compounds showed potent human acrosin inhibitory activity.

2. Chemistry

The synthetic route of the target compounds is outlined in Schemes 1–4. In general, the target compounds were obtained via a two-step strategy. First, the heterocyclic scaffolds were constructed by various ring closure reactions. Then, various side chains were substituted on the scaffold to afford the target compounds [15]. Benzoimidazol-2-amine **1** reacted with dimethyl carbonate in the presence of tetrabutylammonium bromide under reflux to afford the carbamate intermediate **2**. Chlorosulfonyl benzoimidazole **3** was readily prepared by the reaction of chlorosulfonic acid and compound **2**. The reaction of intermediate **3** with different benzyl amines or aliphatic amines in the presence of triethylamine (Et_3N) gave compounds **4**, which were subsequently treated with various alkanolamines in pyridine to afford the target compounds **5a**–**i**. 2-Amino-5-bromobenzothiazole **7** [16] was synthesized according to the method of Gourley [17]. Then, it was treated with 2-chloroacetyl chloride in the presence of Et_3N and THF to afford intermediate **8**. Target compounds **9a**–**e** were obtained by reacting compound **8** with various amines in the presence of Et_3N and DMF. Claisen condensation of 1-(4-methoxyphenyl)ethanone (**10**) with diethyl oxalate in the presence of Na and ethanol afforded compound **11**, which was subsequently reacted with NH_2NH_2 to give phenylpyrazole **12**. Then, compounds **13a**–**d** were obtained by substitution of the acetate group of compound **12** by various alkanolamines. 1*H*-indazole-3-carboxylic acid (**14**) was reacted with ethanol and methylamine in the presence of EDC·HCl, oxalyl dichloride and Et_3N to give compounds **15**. Free amine of compounds **15** was converted to sodium salt, and then treated with 2, 4-dichlorobenzoyl chloride in THF to afford target compounds **17a**–**b**.

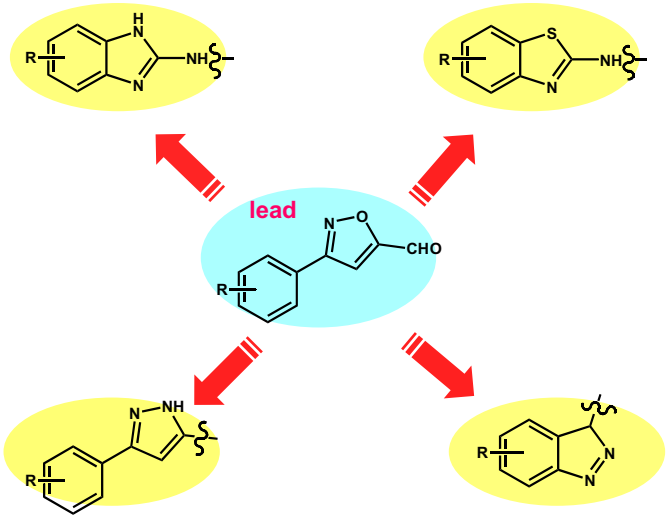


Fig. 2. Scaffold hopping of the isoxazolecarbaldehyde human acrosin inhibitors.

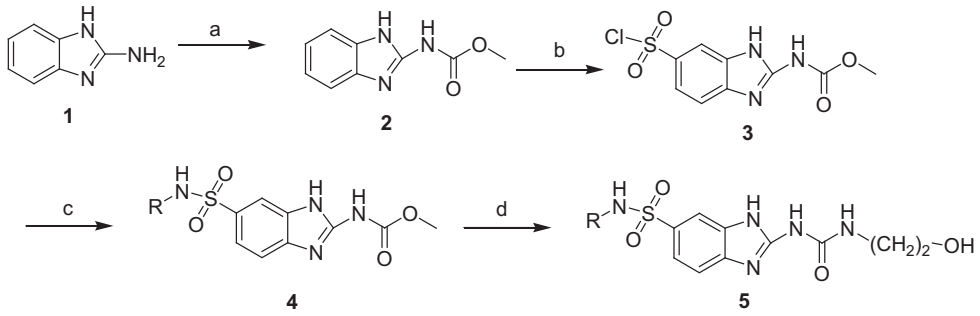
3. Pharmacology

The *in vitro* acrosin inhibitory activities of the target compounds were evaluated by a modification of the method of Kennedy et al. [18], and the detailed protocols can be found in our previous studies [12–14].

4. Results and discussion

4.1. Design rationale

Previous molecular modeling studies indicated that the active site of human acrosin can be divided into three subsites (P1, P2 and G) [10]. Docking model of the isoxazolecarbaldehyde inhibitor with human acrosin revealed that the ligand was located in the P1 pocket, which was a hydrophobic hydrogen-bonding site for inhibitor binding [10]. Its aldehyde oxygen atom formed a hydrogen bond with Thr216. Based on the binding mode, a series of benzoheterocyclic derivatives were rationally designed. First, scaffold hopping was used to replace the isoxazole core by benzoimidazole, benzothiazole, pyrazole, and indazole in order to form stronger hydrophobic and hydrogen bonding interactions with the P1 pocket. Second, the chemically unstable aldehyde group was removed. Its hydrogen bonding interaction with Thr216 could be compensated by the hydrogen bonds between the scaffold or side chain and the P1 pocket. Third, various side chains were introduced



Scheme 1. Reagents and conditions: (a) dimethyl carbonate, tetrabutylammonium bromide, calcium chloride, reflux, 90–100 °C, 6 h, yield 72.3%; (b) chlorosulfonic acid, 0 °C, ethyl acetate, yield 90.2%; (c) tetrahydrofuran, triethylamine, amine derivatives, rt, overnight, yield 50.2–70.3%; (d) ethanolamine, pyridine, reflux, 90–100 °C, 3 h, yield 70.3–85.1%.

on the benzoheterocyclic scaffold to interact with the residues in the P2 or G pocket, which was important to improve the binding affinity of the ligands. As a result, compounds **5a**–**i**, **9a**–**d**, **13a**–**d** and **17a**–**b** were synthesized and assayed.

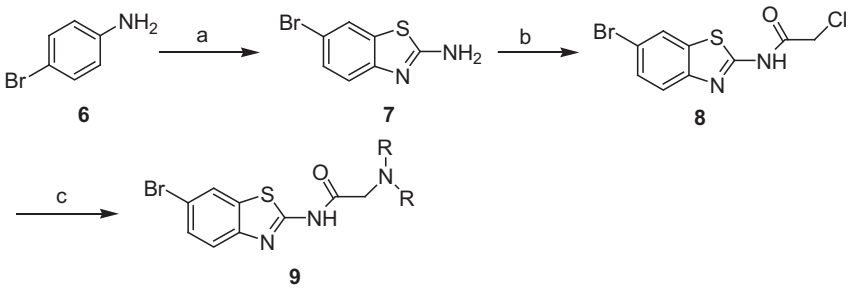
4.2. Human acrosin inhibitory activities and SARs

As shown in Table 1, imidazole derivatives **5a**–**i** generally showed moderate to good human acrosin inhibitory activities with IC_{50} values ranging from 0.08 mM to 7.32 mM. Notably, most of them were more potent than the standard reference drug TLCK (IC_{50} = 142.6 mM) and the isoxazolecarbaldehyde lead (IC_{50} = 39 mM). It was observed that the type and position of the substitutions played an important role for the inhibitory activities. As compared with the unsubstituted benzyl derivative **5g** (IC_{50} = 0.28 mM), the alkyl derivative **5i** showed decreased activity (IC_{50} = 1.62 mM). On the other hand, the introduction of a fluorine atom on the *ortho*-position of the benzyl group (compound **5a**) led to the substantial increase of the inhibitory activities. The IC_{50} value of compound **5a** was 0.08 mM, which was far more potent than TLCK and the isoxazolecarbaldehyde lead. The replacement of the fluorine atom of compound **5a** by a chlorine atom (compound **5h**, IC_{50} = 1.06 mM) or methoxyl group (Compound **5e**, IC_{50} = 6.04 mM) resulted in the decreased activity. Two *meta*-substituted derivatives **5b** and **5f** were moderately active. In contrast, 3, 5-disubstituted derivatives **5c** and **5d** showed increased inhibitory activity. Moreover, the 3, 5-difluoro derivative **5d** (IC_{50} = 0.34 mM) was more potent than the 3, 5-dichloro derivative **5c** (IC_{50} = 2.45 mM), but it was less active than the unsubstituted compound **5g**.

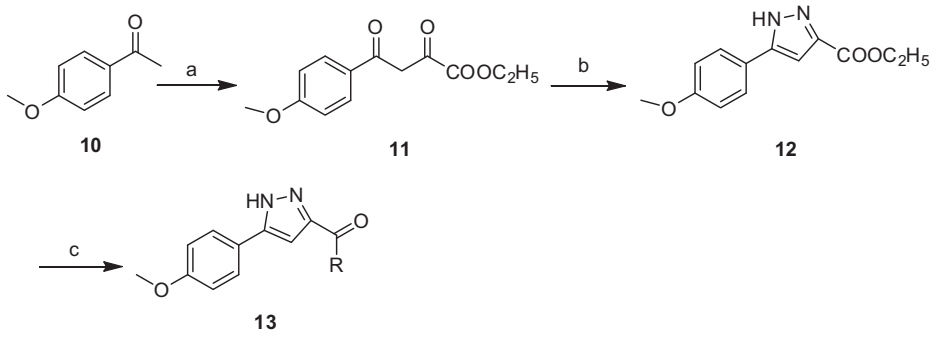
For the thiazole derivatives **9a**–**d**, their IC_{50} values were in the range of 0.54–5.08 mM. Analysis of the SAR of the *N*-substitutions revealed that the pentyl group was the most favorable. When the pentyl group of compound **9c** was replaced by butyl (compound **9a**) or isopentyl (compound **9b**), the acrosin inhibitory activity was decreased by about 5 fold and 2 fold, respectively. As compared with the alkyl derivatives **9a**–**c**, compound **9d**, bearing a methyl piperazinyl substitution, showed obviously decreased inhibitory activity. Phenylpyrazole derivatives **13a**–**d** generally showed moderate acrosin inhibitory activity (IC_{50} range: 1.43–8.92 mM). 3-Hydroxypropyl group was found to be more favorable for the side chain amine substitution than the 2-hydroxypropyl (compound **13d**) or 3-hydroxyethyl (compounds **13a** and **13**b) group. When the phenylpyrazole scaffold was replaced by indazole (compounds **17a** and **17b**), similar inhibitory activity was observed.

4.3. The binding modes of the new human acrosin inhibitors

In order to obtain the binding modes of the benzoheterocyclic inhibitors, representative compounds **5a**, **9c**, **13a** and **17a** were docked into the active site of human acrosin. As shown in Fig. 3A, compound **5a** bound with the active site of human acrosin mainly through hydrophobic and hydrogen bonding interactions. Its benzoimidazole core and C2-side chain was located in the P1 pocket. The urea carbonyl group, amine group and terminal hydroxyl group formed three hydrogen bonds with Gln218, Trp243 and Val245, respectively. Moreover, the benzyl sulfonamide group of compound **5a** was extended to the G pocket and formed hydrophobic interactions. The substitution on the terminal phenyl could interact with Arg199. For example, the difluoro derivative **5d** showed better activity than the dichloro derivative **5c** because it formed stronger electrostatic interaction with Arg199. Compound **13a** showed a similar binding mode (Fig. 3C) to that of compound **5a**. The pyrazole core and amide side chain interacted with the P1 pocket. The pyrazole nitrogen atom and terminal hydroxyl group formed hydrogen bonding interactions with Ser221 and Thr216, respectively. The methoxyl phenyl group of compound **13a** mimicked the benzyl group of compound **5a** and formed similar hydrophobic interaction with the G pocket. In contrast, two pentyl groups of compound **9c** could form hydrophobic interactions with the P2 and



Scheme 2. Reagents and conditions: (a) 1.1 equiv of NH_4NCS and 1.1 equiv of benzoyl chloride in acetone, reflux 1.5 h, then 1 equiv of Br_2 in dichloroethane <32 °C for 18 h, yield 65.2%; (b) 2-chloroacetyl chloride, triethylamine, THF, rt, 1 h, yield 90%; (c) various amines, DMF, triethylamine, reflux, 85 °C, 3–5 h, yield 45.2–67.3%.



Scheme 3. (a) Diethyl oxalate, ethanol, Na, rt, 8 h, yield 70%; (b) NH_2NH_2, ethanol, reflux, 80–90 °C, 4–8 h, yield 78.2%; (c) various alkanolamines, pyridine, reflux, 90–100 °C, 3 h, yield 70.2–89.1%.

G pocket (Fig. 3B), respectively. Thus, compound **9c** showed better inhibitory activity than the butyl derivative **9a** because it formed stronger hydrophobic interaction with acrosin. Moreover, its amide NH formed a hydrogen bond with the backbone of Val245. The indazole derivative **17a** formed similar interactions with the P1 and G pocket (Fig. 3D) with its *N*-carbonyl group and ester group forming hydrogen bonding interaction with Gln218 and His69, respectively. The binding mode of the reference drug TLCK with human acrosin has been investigated in our previous studies [11]. TLCK could also interact with the P1, P2 and G pocket, but it formed relatively weak hydrophobic interactions. Thus, the designed compounds showed higher inhibitory activities.

5. Conclusion

In summary, scaffold hopping was used to optimize the isoxazolecarbaldehyde human acrosin inhibitors. A series of benzoimidazole, benzothiazole, pyrazole, and indazole derivatives were rationally designed and synthesized. Most of them showed moderate to good human acrosin inhibitory activities. Molecular docking studies revealed that the heterocyclic inhibitors formed hydrophobic and hydrogen bonding interactions with the active site of human acrosin. The benzoimidazole derivative **5a** was far more potent than the standard reference drug TLCK, which represents a good lead structure to develop male contraceptives.

6. Experimental protocols

6.1. General procedure for the synthesis of compounds

Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker 300 spectrometer with TMS as an internal standard and $CDCl_3$/DMSO as solvent. Chemical shifts (δ values) and coupling constants (J values) are given in ppm and Hz, respectively. ESI mass spectra were performed on an API-3000 LCeMS spectrometer. TLC analysis was carried out on silica gel plates GF254 (Qindao Haiyang Chemical, China). Silica gel column chromatography was performed with Silica gel 60G (Qindao Haiyang Chemical, China). Commercial solvents were used without any pretreatment.

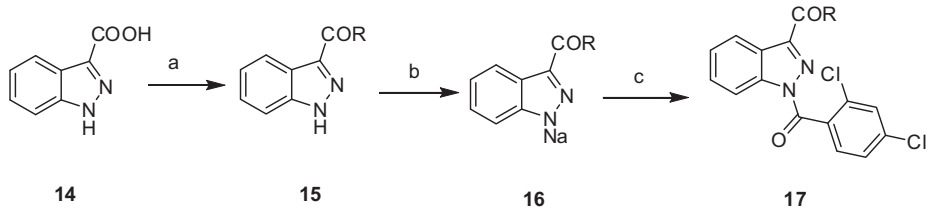
6.1.1. Chemical synthesis of N-(2-fluorobenzyl)-2-(3-(2-hydroxyethyl)ureido)-1H-benzo[d]imidazole-5-sulfonamide (*5a*, Scheme 1)

A solution of compound **4** (0.5 g, 1.38 mmol) and 2-aminoethanol (3.04 g, 50 mmol) in pyridine (15 mL) was stirred under reflux for 3 h. Then, the mixture was stirred at room temperature for 10 h. The solid was filtrated and was purified by column chromatography (CH_2Cl_2:MeOH = 60:2, v/v) to give **5a** as white solid (0.48 g, yield 90%). M.p. 198.3–198.9 °C ^{1}H NMR (300 MHz, DMSO) δ 10.72 (s, 2H, NH), 7.80 (s, 1H, Ar-H), 7.47 (s, 2H, Ar-H), 7.33 (s, 1H, Ar-H), 7.23 (d, 1H, J = 7.1 Hz, Ar-H), 7.15–7.02 (m, 2H, Ar-H), 4.84 (s, 1H, OH), 3.94 (s, 2H, $PhCH_2$), 3.49–3.47 (m, 2H, urea-$\underline{CH_2}$), 3.28–3.24 (m, 2H, $\underline{CH_2}$-OH). MS (ESI) m/z: 408 (M + 1). ^{13}C NMR (75 MHz, DMSO) δ 161.48, 158.23, 154.03, 150.65, 131.89, 130.14, 129.28, 129.17, 124.78, 124.59, 124.19, 119.29, 115.12, 114.83, 59.98, 41.93, 41.93. HRMS: m/z (%): 408.1132 $[M + H]^+$

The synthetic procedure for the target compounds **5b–i** and **13a–d** was similar to the synthesis of compound **5a**.

6.1.2. Chemical synthesis of N-(6-bromobenzo[d]thiazol-2-yl)-2-(dibutylamino) acetamide (*9a*, Scheme 2)

Et_3N (3 drops) was added to a solution of compound **8** (1.0 g, 3.3 mmol) and dibutylamine (0.51 g, 3.96 mmol) in DMF (15 mL). The mixture was refluxed for 6 h, then diluted with H_2O (20 mL) and stirred at room temperature for 12 h. The solid was filtrated and was purified by column chromatography (CH_2Cl_2:MeOH = 100:1, v/v) to give **9a** as white solid (1.1 g, yield 85%). M.p. 69.2–69.4 °C ^{1}H NMR (300 MHz, DMSO) δ 8.25–8.24 (m, 1H, Ar-H), 7.67 (d, 1H, J = 8.6 Hz, Ar-H), 7.58–7.57 (m, 1H, Ar-H), 3.42 (s, 2H, $NHCO\underline{CH_2}$), 2.58–2.51 (m, 4H, 2 × $N\underline{CH_2}CH_2CH_2$), 1.50–1.32 (m, 4H, 2 × $NCH_2\underline{CH_2}CH_2$), 1.32–1.20 (m, 4H, 2 × $NCH_2CH_2\underline{CH_2}$), 0.85 (t, J = 7.3 Hz, 6H, 2 × CH_3). MS (ESI) m/z: 398 (M + 1). ^{13}C NMR (75 MHz, DMSO) δ 171.19, 158.38, 147.57, 134.01, 128.99, 124.06, 121.97, 115.35, 56.42, 53.58, 53.58, 28.82, 28.82, 20.13, 20.13, 14.04, 14.04. HRMS: m/z (%): 398.0893 $[M + H]^+$.



Scheme 4. (a) Ethanol or methylamine, EDC·HCl/oxalyl dichloride, Et_3N, reflux, 90–100 °C, 1–10 h, yield 85–95%; (b) THF, 60% NaH, 0–5 °C, 1–3 h, yield 81–88%; (c) 2, 4-dichlorobenzoyl chloride, THF, rt, 0–5 °C, 1–3 h, yield 71–80%.

Table 1
Structures and human acrosin inhibitory activities of the target compounds.

Compound	R	Formula	IC_{50} (mM)
5a	R = (2-F)$PhCH_2$–	$C_{17}H_{18}FN_5O_4S$	0.08
5b	R = (3-OCH_3)$PhCH_2$–	$C_{18}H_{21}N_5O_5S$	7.32
5c	R = (3,5-Cl)$PhCH_2$–	$C_{17}H_{17}Cl_2N_5O_4S$	2.45
5d	R = (3,5-F)$PhCH_2$–	$C_{17}H_{17}F_2N_5O_4S$	0.34
5e	R = (2-OCH_3)$PhCH_2$–	$C_{18}H_{21}N_5O_5S$	6.04
5f	R = (3-CH_3)$PhCH_2$–	$C_{18}H_{21}N_5O_4S$	5.45
5g	R = $PhCH_2$–	$C_{17}H_{19}N_5O_4S$	0.28
5h	R = (2-Cl)$PhCH_2$–	$C_{17}H_{18}ClN_5O_4S$	1.06
5i	R = $CH_3(CH_2)_5$–	$C_{15}H_{23}N_5O_4S$	1.62
9a	R = $CH_3(CH_2)_3$–	$C_{17}H_{24}BrN_3OS$	2.78
9b	R = $(CH_3)_2CH(CH_2)_2$–	$C_{19}H_{28}BrN_3OS$	1.09
9c	R = $CH_3(CH_2)_4$–	$C_{19}H_{28}BrN_3OS$	0.54
9d	R = –$(CH_2)_2N[(CH_2)_4CH_3](CH_2)_2$–	$C_{17}H_{23}BrN_4OS$	5.08
13a	R = –$N(CH_3)CH_2CH_2OH$	$C_{14}H_{17}N_3O_3$	6.81
13b	R = –$N(CH_2CH_2OH)_2$	$C_{15}H_{19}N_3O_4$	5.73
13c	R = –$NHCH_2CH_2CH_2OH$	$C_{14}H_{17}N_3O_3$	1.43
13d	R = –$NHCH_2CH(OH)CH_3$	$C_{14}H_{17}N_3O_3$	8.92
17a	R = –OCH_2CH_3	$C_{17}H_{12}Cl_2N_2O_3$	3.24
17b	R = –$NHCH_3$	$C_{16}H_{11}Cl_2N_3O_2$	3.78
TLCK			142.6

The synthetic procedure for the target compounds **9b–d** was similar to the synthesis of compound **9a**.

*6.1.3. Chemical synthesis of ethyl 1-(2, 4-dichlorobenzoyl)-1H-indazole-3-carboxylate (**17a**, Scheme 4)*

2, 4-Dichlorobenzoyl chloride (2.07 g, 0.01 mol) in THF (5 mL) was added dropwise to a solution of compound **16** (2.12 g, 0.01 mol) in THF (10 mL). The mixture was stirred at 0–5 °C for 2–6 h. After removing THF, ice water (10 mL) was added to the residue, and stirred at room temperature. The solid was filtrated and was purified by recrystallization to give **17a** as white solid (2.72 g, 75%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.58 (d, J = 9.0 Hz, 1H, Ar-H), 8.26 (d, J = 9.0 Hz, 1H, Ar-H), 7.73–7.67 (m, 1H, Ar-H), 7.57–7.53 (m, 3H, Ar-H), 7.41 (dd, J = 9.0, 3.0 Hz, 1H, Ar-H), 4.50 (q, J = 7.2 Hz, 2H, CH_2), 1.46 (t, J = 6.0 Hz, 3H, CH_3). MS (ESI) m/z: 363 (M + 1). ^{13}C NMR (75 MHz, DMSO) δ 166.12, 160.67, 141.83, 139.90, 136.37, 132.45, 131.65, 131.49, 130.74, 129.25, 127.55, 126.75, 124.30, 122.40, 115.06, 61.74, 14.05. HRMS: m/z (%): 363.0292 $[M + H]^+$.

*6.1.4. 2-(3-(3-Hydroxyethyl)ureido)-N-(2-methoxybenzyl)-1H-benzo[d]imidazole-5-sulfonamide (**5b**)*

^{1}H NMR (300 MHz, DMSO) δ 11.94 (s, 1H), 10.13 (s, 1H), 7.91 (t, J = 4.3 Hz, 1H), 7.81 (s, 1H), 7.50–7.48 (m, 2H), 7.19–7.13 (m, 2H), 6.80–6.74 (m, 3H), 4.84 (s, 1H), 3.88 (d, J = 5.6 Hz, 2H), 3.65 (s, 3H), 3.51–3.46 (m, 2H), 3.27–3.22 (m, 2H). MS (ESI) m/z: 420 (M + 1).

*6.1.5. N-(3,5-Dichlorobenzyl)-2-(3-(2-hydroxyethyl)ureido)-1H-benzo[d]imidazole-5-sulfonamide (**5c**)*

^{1}H NMR (300 MHz, DMSO) δ 11.07 (s, 1H), 10.25 (s, 1H), 8.02 (s, 1H), 7.79 (s, 1H), 7.52 (d, J = 2.1 Hz, 1H), 7.48 (s, 2H), 7.46–7.40 (m, 1H), 7.37–7.33 (m, 1H), 7.20–7.15 (m, 1H), 4.85 (s, 1H), 3.97 (s, 2H), 3.48 (s, 2H), 3.28–3.22 (m, 2H). MS (ESI) m/z: 458 (M + 1).

*6.1.6. N-(3, 5-Difluorobenzyl)-2-(3-(2-hydroxyethyl)ureido)-1H-benzo[d]imidazole-5-sulfonamide (**5d**)*

^{1}H NMR (300 MHz, DMSO) δ 12.00 (s, 1H), 10.13 (s, 1H), 7.96 (s, 1H), 7.84–7.71 (m, 1H), 7.46 (s, 2H), 7.35 (dd, J = 15.6, 8.7 Hz, 1H), 7.15–7.07 (m, 2H), 6.98 (t, J = 8.2 Hz, 1H), 4.85 (s, 1H), 3.92 (d, J = 5.8 Hz, 2H), 3.51–3.45 (m, 2H), 3.28–3.23 (m, 2H). MS (ESI) m/z: 426 (M + 1).

*6.1.7. 2-(3-(2-Hydroxyethyl)ureido)-N-(2-methoxybenzyl)-1H-benzo[d]imidazole-5-sulfonamide (**5e**)*

^{1}H NMR (300 MHz, DMSO) δ 11.98 (s, 1H), 10.22 (s, 1H), 7.81 (s, 1H), 7.77–7.73 (m, 1H), 7.48 (s, 2H), 7.25 (d, J = 7.6 Hz, 1H), 7.21 (s, 1H), 7.17 (d, J = 8.2 Hz, 1H), 6.88–6.83 (m, 2H), 4.84 (s, 1H), 3.85 (d, J = 5.6 Hz, 2H), 3.67 (s, 3H), 3.51–3.46 (m, 2H), 3.27–3.22 (m, 2H). MS (ESI) m/z: 420 (M + 1).

*6.1.8. 2-(3-(2-Hydroxyethyl)ureido)-N-(2-methylbenzyl)-1H-benzo[d]imidazole-5-sulfonamide (**5f**)*

^{1}H NMR (300 MHz, DMSO) δ 11.91 (s, 1H), 10.15 (s, 1H), 7.87 (t, J = 5.8 Hz, 1H), 7.81 (s, 1H), 7.48 (s, 2H), 7.15–7.10 (m, 2H), 6.99 (d, J = 8.3 Hz, 3H), 4.84 (s, 1H), 3.86 (d, J = 6.1 Hz, 2H), 3.51–3.46 (m, 2H), 3.27–3.22 (m, 2H), 2.19 (s, 3H). MS (ESI) m/z: 404 (M + 1). ^{13}C NMR (75 MHz, DMSO) δ 154.03, 150.47, 137.85, 137.74, 137.21, 128.18, 128.18, 128.06, 128.06, 127.62, 127.62, 124.67, 124.67, 119.47, 59.99, 46.20, 41.97, 20.89. HRMS: m/z (%): 404.1385 $[M + H]^+$.

*6.1.9. N-Benzyl-2-(3-(2-hydroxyethyl)ureido)-1H-benzo[d]imidazole-5-sulfonamide (**5g**)*

^{1}H NMR (300 MHz, DMSO) δ 11.99 (s, 1H), 10.13 (s, 1H), 7.94–7.80 (m, 2H), 7.49 (s, 2H), 7.25–7.21 (m, 5H), 7.14 (s, 1H), 4.85 (s, 1H), 3.90 (d, J = 6.2 Hz, 2H), 3.51–3.46 (m, 2H), 3.27–3.22 (m, 2H). MS (ESI) m/z: 390 (M + 1).

*6.1.10. N-(2-Chlorobenzyl)-2-(3-(2-hydroxyethyl)ureido)-1H-benzo[d]imidazole-5-sulfonamide (**5h**)*

^{1}H NMR (300 MHz, DMSO) δ 10.60 (s, 2H), 7.81 (s, 1H), 7.51–7.48 (m, 2H), 7.44–7.40 (m, 1H), 7.38–7.35 (m, 1H), 7.28–7.21 (m, 3H), 4.84 (s, 1H), 3.98 (s, 2H), 3.51–3.45 (m, 2H), 3.27–3.22 (m, 2H). MS (ESI) m/z: 424 (M + 1).

*6.1.11. N-Hexyl-2-(3-(2-hydroxyethyl)ureido)-1H-benzo[d]imidazole-5-sulfonamide (**5i**)*

^{1}H NMR (300 MHz, DMSO) δ 11.94 (s, 1H), 10.13 (s, 1H), 7.77 (s, 1H), 7.46 (s, 2H), 7.32 (t, J = 5.9 Hz, 1H), 7.19–7.13 (m, 1H), 4.84 (s, 1H), 3.48 (q, J = 9.7, 4.8 Hz, 2H), 3.24 (q, J = 11.0, 5.5 Hz, 2H), 2.65 (q, J = 13.0, 6.7 Hz, 2H), 1.34–1.25 (m, 2H), 1.19–1.12 (m, 6H), 0.78 (t, J = 6.8 Hz, 3H). MS (ESI) m/z: 384 (M + 1).

*6.1.12. N-(5-Bromobenzo[d]thiazol-2-yl)-2-(diisopentylamino) acetamide (**9b**)*

^{1}H NMR (300 MHz, DMSO) δ 10.46 (s, 1H), 7.94 (d, J = 1.9 Hz, 1H), 7.65 (d, J = 8.6 Hz, 1H), 7.53 (dd, J = 8.6, 1.6 Hz, 1H), 3.23 (s, 2H), 2.61–2.56 (m, 4H), 1.65–1.51 (m, 2H), 1.42–1.25 (m, 4H), 0.90 (d, J = 6.6 Hz, 12H). MS (ESI) m/z: 426 (M + 1). ^{13}C NMR (75 MHz, DMSO) δ 170.81, 158.08, 147.43, 133.56, 128.90, 124.42, 121.96, 115.35, 56.74, 51.96, 51.96, 35.38, 35.38, 25.72, 25.72, 22.27, 22.27, 22.27, 22.27. HRMS: m/z (%): 426.121$[M + H]^+$.

*6.1.13. N-(5-Bromobenzo[d]thiazol-2-yl)-2-(dipentylamino) acetamide (**9c**)*

^{1}H NMR (300 MHz, DMSO) δ 10.22 (s, 1H), 7.94 (d, J = 1.9 Hz, 1H), 7.65 (d, J = 8.6 Hz, 1H), 7.53 (dd, J = 8.6, 2.0 Hz, 1H), 3.31 (s, 2H), 2.57 (t, J = 15.4 Hz, 4H), 1.55–1.47 (m, 4H), 1.33–1.25 (m, 8H), 0.89 (t, J = 6.7 Hz, 6H). MS (ESI) m/z: 426 (M + 1).

*6.1.14. N-(5-Bromobenzo[d]thiazol-2-yl)-2-(4-pentylpiperazin-1-yl)acetamide (**9d**)*

^{1}H NMR (300 MHz, DMSO) δ 12.29 (s, 1H), 8.26 (d, J = 2.1 Hz, 1H), 7.67 (d, J = 8.6 Hz, 1H), 7.57 (dd, J = 8.6, 2.1 Hz, 1H), 3.42 (s, 2H), 3.38–3.30 (m, 8H), 2.71 (m, 2H), 1.54 (s, 2H), 1.32–1.21 (m, 4H), 0.86 (t, J = 6.9 Hz, 3H). MS (ESI) m/z: 426 (M + 1).

*6.1.15. N-(2-Hydroxyethyl)-5-(4-methoxyphenyl)-N-methyl-1H-pyrazole-3-carboxamide (**13a**)*

^{1}H NMR (600 MHz, DMSO) δ 13.43 (s, 1H, NH), 7.71 (d, J = 8.7 Hz, 2H, Ar-H), 7.03 (d, J = 8.7 Hz, 2H, Ar-H), 6.98 (d, J = 8.7 Hz, 1H, CH),

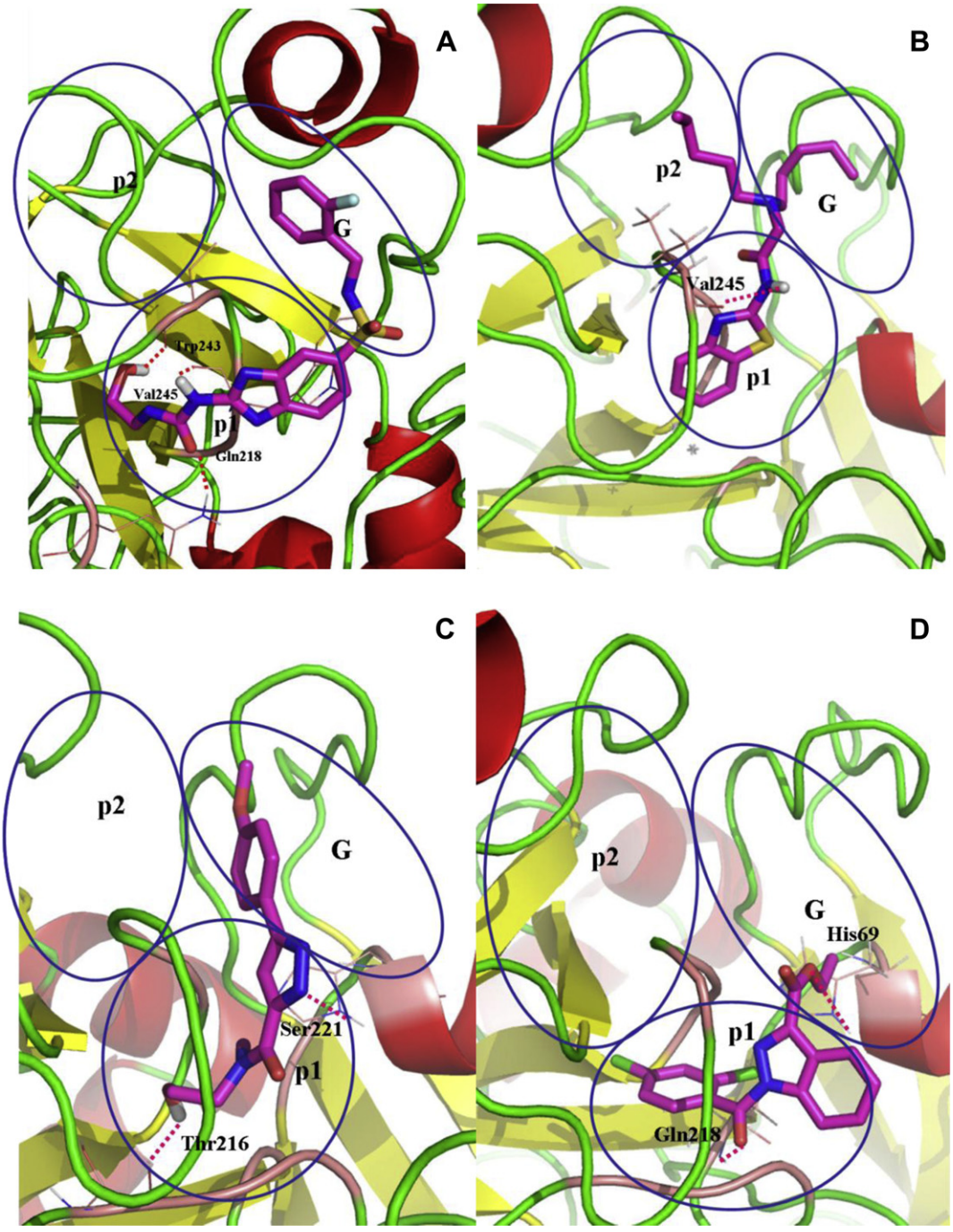


Fig. 3. The binding modes of compounds **5a** (A), **9c** (B), **13a** (C) and **17a** (D) with the active site of human acrosin.

4.75 (s, 1H, OH), 3.77 (d, J = 6.0 Hz, 2H, CH_2OH), 3.61 (q, J = 8.4 Hz, 2H, NCH_2), 3.02 (s, 3H, CH_3). MS (ESI) m/z: 276 (M + 1).

*6.1.16. N,N-bis(2-Hydroxyethyl)-5-(4-methoxyphenyl)-1H-pyrazole-3-carboxamide (**13b**)*

^{1}H NMR (300 MHz, DMSO) δ 13.46 (s, 1H), 7.71 (d, J = 8.3 Hz, 2H), 7.01 (d, J = 8.2 Hz, 2H), 6.87 (s, 1H), 4.79 (s, 2H), 3.80 (s, 2H), 3.78 (s, 3H), 3.60 (d, J = 5.6 Hz, 4H), 3.54 (d, J = 4.8 Hz, 2H). MS (ESI) m/z: 306 (M + 1).

*6.1.17. N-(3-Hydroxypropyl)-5-(4-methoxyphenyl)-1H-pyrazole-3-carboxamide (**13c**)*

^{1}H NMR (600 MHz, DMSO) δ 13.43 (s, 1H), 8.11 (s, 1H), 7.72 (d, J = 7.0 Hz, 2H), 6.98 (d, J = 54.1 Hz, 3H), 4.50 (s, 1H), 3.80 (s, 3H), 3.47 (q, J = 6.0 Hz, 2H), 3.34–3.26 (m, 2H), 1.67 (s, 2H). MS (ESI) m/z: 276 (M + 1). ^{13}C NMR (75 MHz, DMSO) δ 161.85, 158.96, 148.10, 143.45, 126.38, 126.38, 121.77, 114.81, 114.81, 101.25, 57.33, 54.53, 35.70, 31.62. HRMS: m/z (%): 276.1348 $[M + H]^+$.

*6.1.18. N-(2-Hydroxypropyl)-5-(4-methoxyphenyl)-1H-pyrazole-3-carboxamide (**13d**)*

^{1}H NMR (300 MHz, DMSO) δ 13.47 (s, 1H), 7.90 (s, 1H), 7.72 (d, J = 8.5 Hz, 2H), 7.02 (d, J = 8.5 Hz, 2H), 6.95 (s, 1H), 4.81 (s, 1H), 3.78 (s, 3H), 3.34 (s, 1H), 3.31–3.09 (m, 2H), 1.05 (d, J = 6.0 Hz, 3H). MS (ESI) m/z: 276 (M +1). ^{13}C NMR (75 MHz, DMSO) δ 161.84, 159.42, 147.75, 143.53, 126.78, 126.35, 121.47, 114.42, 114.42, 101.68, 65.28, 55.17, 46.18, 21.14. HRMS: m/z (%): 236.1343 $[M + H]^+$.

*6.1.19. 1-(2, 4-Dichlorobenzoyl)-N-methyl-1H-indazole-3-carboxamide (**17b**)*

^{1}H NMR (300 MHz, $CDCl_3$) δ 10.30 (s, 1H), 8.43 (d, J = 8.2 Hz, 1H), 7.52 (d, J = 8.1 Hz, 1H), 7.47–7.41 (m, 1H), 7.33–7.30 (m, 3H), 7.06 (s, 1H), 3.07 (d, J = 5.0 Hz, 3H). MS (ESI) m/z: 348 (M + 1). ^{13}C NMR (75 MHz, DMSO) δ 162.71, 162.71, 140.96, 140.96, 138.37, 138.37, 126.28, 126.28, 121.81, 121.81, 121.52, 121.52, 121.34, 110.46, 110.46, 25.14.

6.2. Molecular docking

In our previous studies, Libdock within the Discovery Studio 2.5 [19] software package was found to be a good method to dock human acrosin inhibitors [13]. The protein structure of human acrosin was obtained from our previous report [19]. In the process of Libdock docking, the docking preferences parameter was set to “high quality” and the conformation method was set to “best”. Other parameters were used as default.

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