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Synthesis and acrosin inhibitory activity of methyl 5-substituted-1*H*-benzo[*d*]imidazol-2-yl carbamate derivatives

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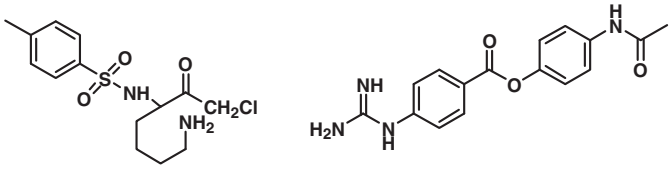
ABSTRACT

A series of novel methyl 5-substituted 1*H*-benzo[*d*]imidazol-2-ylcarbamates were designed, synthesized, and their acrosin inhibitory activities evaluated in vitro. The results of acrosin inhibitory activity showed that all title compounds were more potent than the control TLCK. Compound **4w** displayed the most potent acrosin inhibitory activity among all the compounds, with an IC_{50} of 6.3×10^{-5} M. The studies provide a new structural class for the development of novel acrosin inhibitory agents.

The contraceptive pill is one of the most important methods of contraception, protecting millions of women worldwide from unwanted pregnancies therefore allowing a measure of control over reproduction. However, the oral contraceptive pill is only available for women and most of these pills can have serious side effects, such as breakthrough menstrual bleeding, nausea, vomiting, acne, breast tenderness, cramping, weight gain, heavy bleeding and headaches.[1–4] Therefore, there is a need for new contraceptive agents and methods which are easier to use with fewer side effects and available for both men and women.



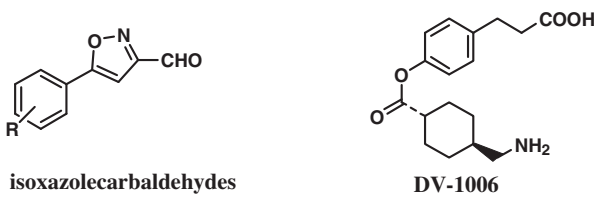


Figure 1. Chemical structures of TLCK, AGB, DV-1006, and substituted isoxazolecarbaldehydes.

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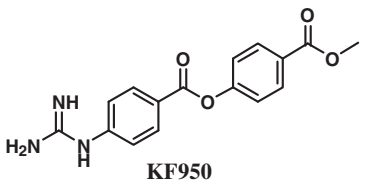


Figure 2. Chemical structures of KF-950.

Sperm acrosin is the major trypsin-like protease present in the acrosome of all mammalian spermatozoa,[5] and is also a sperm-specific enzyme involved in the acrosome reaction. Acrosin plays a critical role in fertilization where it hydrolyzes the zona pellucida and the vitelline membrane, and facilitates the penetration of the sperm through the innermost glycoprotein layers of the oocytes.[6] Inhibition of acrosin activity should block fertilization and/or reduce the success of fertilization.[7] Therefore, acrosin is a key enzyme in fertilization, and is also a potential target for the design and development of novel male contraceptive agents. In recent years, several acrosin inhibitors have been reported such as *N*-alpha-tosyl-*L*-lysyl-chloromethyl-ketone (TLCK), DV-1006, isoxazolecarbaldehydes and 4-guanidinobenzoates (Fig. 1).[8–11]

In recent years, our group has focused on the research of novel acrosin inhibitors. In a previous study, we constructed a homologous three-dimensional model of human acrosin based on the crystal structures of the ram and boar forms,[12] since no crystal structures of human acrosin have been reported. The active site

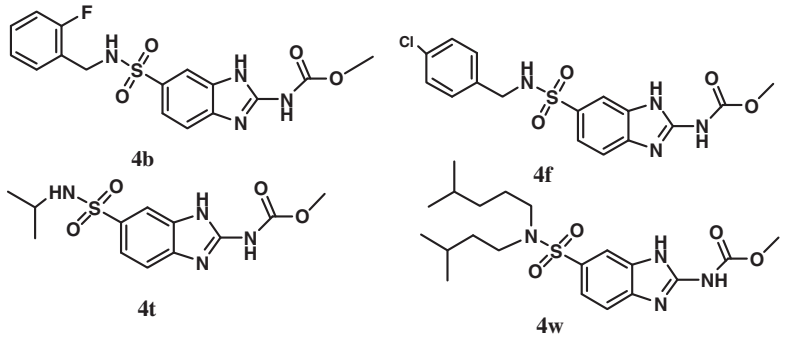
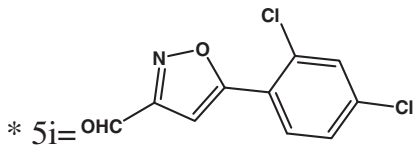
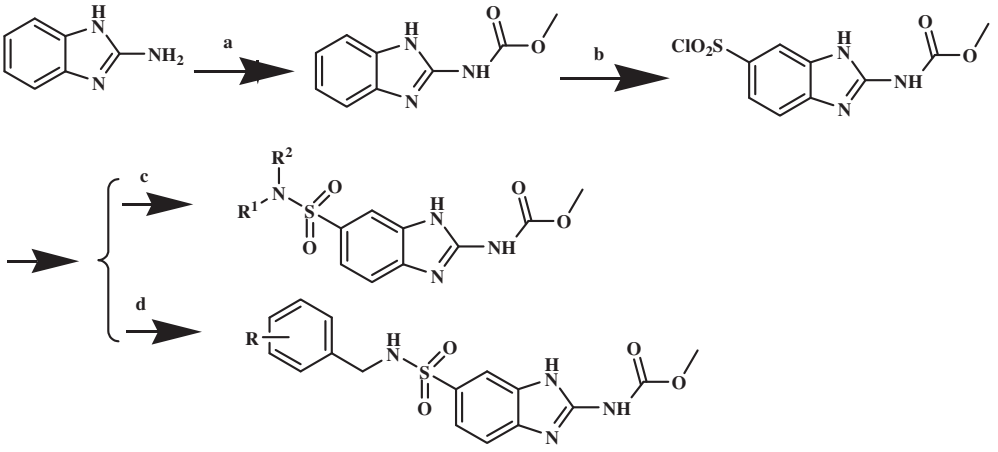


Figure 3. Chemical structures of the target compounds.

Table 1
Acrosin inhibitory property of the target compounds

Compound	R	Formula	IC_{50} (mM)
4a	R_1 = (2, 5-CH_3)Ph–, R_2 = H	$C_{17}H_{18}N_4O_4S$	8.86
4b	2-F	$C_{16}H_{15}FN_4O_4S$	0.31
4c	4-F	$C_{16}H_{15}FN_4O_4S$	2.40
4d	4-Br	$C_{16}H_{15}BrN_4O_4S$	1.64
4e	2-Cl	$C_{16}H_{15}N_4O_4SCl$	5.75
4f	4-Cl	$C_{16}H_{15}N_4O_4SCl$	7.52
4g	2, 4-Cl	$C_{16}H_{14}N_4O_4SCl_2$	1.27
4h	3, 4-Cl	$C_{16}H_{14}N_4O_4SCl_2$	4.02
4i	4-OCH_3	$C_{17}H_{18}N_4O_5S$	3.29
4j	3-CH_3	$C_{17}H_{18}N_4O_4S$	5.06
4k	4-CH_3	$C_{17}H_{18}N_4O_4S$	3.18
4l	H	$C_{16}H_{15}N_4O_4S$	1.03
4m	4-CF_3	$C_{17}H_{15}F_3N_4O_4S$	10.70
4n	2, 4-F	$C_{16}H_{14}F_2N_4O_4S$	1.55
4o	3, 4-F	$C_{16}H_{14}F_2N_4O_4S$	1.59
4p	3-F	$C_{16}H_{15}FN_4O_4S$	1.81
4q	2-OCH_3	$C_{17}H_{18}N_4O_5S$	8.42
4r	3-OCH_3	$C_{17}H_{18}N_4O_5S$	7.50
4s	3,4-OCH_3	$C_{18}H_{20}N_4O_6S$	11.50
4t	R_1 = $(CH_3)_2CH$–, R_2 = H	$C_{12}H_{16}N_4O_4S$	0.47
4u	R_1 = $CH_3(CH_2)_3$–, R_2 = H	$C_{13}H_{18}N_4O_4S$	1.77
4v	R_1 = $CH_3(CH_2)_5$–, R_2 = H	$C_{15}H_{22}N_4O_4S$	1.80
4w	R_1 = $(CH_3)_2CH(CH_2)_2$–, R_2 = $(CH_3)_2CH(CH_2)_2$–	$C_{19}H_{31}N_4O_4S$	0.063
4x	R_1 = $PhCH_2$–, R_2 = CH_3–	$C_{17}H_{18}N_4O_4S$	1.25
TLCK			142.6
5-i		$C_{10}H_5Cl_2NO_2$	1.70





Compound	R	Compound	R
4a	R^1 = (2, 5-CH_3)Ph-, R^2 = H	**4m**	4-CF_3
4b	2-F	**4n**	2, 4-F
4c	4-F	**4o**	3, 4-F
4d	4-Br	**4p**	3-F
4e	2-Cl	**4q**	2-OCH_3
4f	4-Cl	**4r**	3-OCH_3
4g	2, 4-Cl	**4s**	3, 4-OCH_3
4h	3, 4-Cl	**4t**	R^1 = $(CH_3)_2CH$-, R^2 = H
4i	4-OCH_3	**4u**	R^1 = $CH_3(CH_2)_3$-, R^2 = H
4j	3-CH_3	**4v**	R^1 = $CH_3(CH_2)_5$-, R^2 = H
4k	4-CH_3	**4w**	R^1 = $(CH_3)_2CH(CH_2)_2$-, R^2 = $(CH_3)_2CH(CH_2)_2$-
4l	H	**4x**	R^1 = $PhCH_2$-, R^2 = CH_3-

Scheme 1. Synthesis of the target compounds **4a**–**4x**. Reagents and conditions: (a) Calcium chloride, tetrabutylammonium bromide, dimethyl carbonate, reflux, 90–100 °C, 6 h, yield 72.3%; (b) chlorosulfonic acid, 0 °C, ethyl acetate, yield 90.2%; (c) synthesis of the target compounds **4a**, **4t**–**4x**: ethyl acetate, triethylamine, 2,5 -dimethyl aniline, rt, overnight; (d) synthesis of the target compounds **4b**–**4s**: tetrahydrofuran, triethylamine, benzyl amine derivatives, rt, overnight.

of human acrosin was analyzed using the multiple copy simultaneous search (MCSS) method,[13,14] which showed that the active site of human acrosin can be divided into three parts: P1 pocket, P2 pocket and G pocket. A parallel virtual screening strategy in combination with pharmacophore-based and docking-based techniques[15] provided a good shortcut for development of acrosin inhibitory agents. We designed and synthesized several novel small acrosin inhibitors based on this model such as KF-950 (Fig. 2), 7-azaindol derivatives,[16] and substituted ethyl 5-(4-aminophenyl)-1*H*-pyrazole-3-carboxylate derivatives.[17]

In this Letter, we report the synthesis and the in vitro acrosin inhibitory activities of novel methyl 5-substituted-1*H*-benzo[*d*] imidazol-2-ylcarbamate compounds (Fig. 3, Table 1). Methyl benzimidazole-2-carbamates (such as Albendazole) are mainly used as antiparasitic drugs affecting the tubulin polymerization in helminths.[18] We have found that methyl benzimidazole-2-carbamates could be a scaffold of acrosin inhibitory by molecular docking.

The chemical synthesis of the target compounds is outlined in Scheme 1. The starting compound, methyl [5-(chlorosulfonyl)-1*H*-benzo[*d*]imidazol-2-yl]-carbamate (**3**) was readily prepared by the reaction of chlorosulfonic acid and methyl 1*H*-benzo[*d*]imidazol-2-yl carbamate (**2**), which can be obtained from commercially available 1*H*-benzo[*d*]imidazol-2-amine (**1**) and dimethyl carbonate in the presence of tetrabutylammonium bromide under reflux. The reaction of intermediate (**3**) with different benzyl amines or aliphatic amines in the presence of triethylamine gave the target

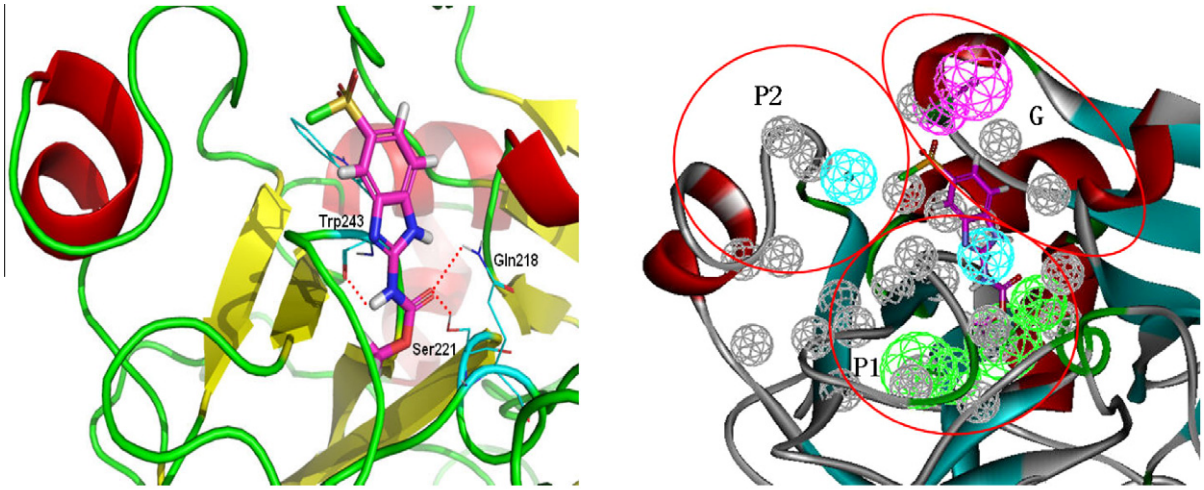


Figure 4. Identification of regions where acrosin protein interacts with various pharmacophores in 1*H*-benzo[*d*]imidazol-2-ylcarbamate and 1*H*-benzo[*d*]imidazol-2-ylcarbamate in the active site of acrosin.

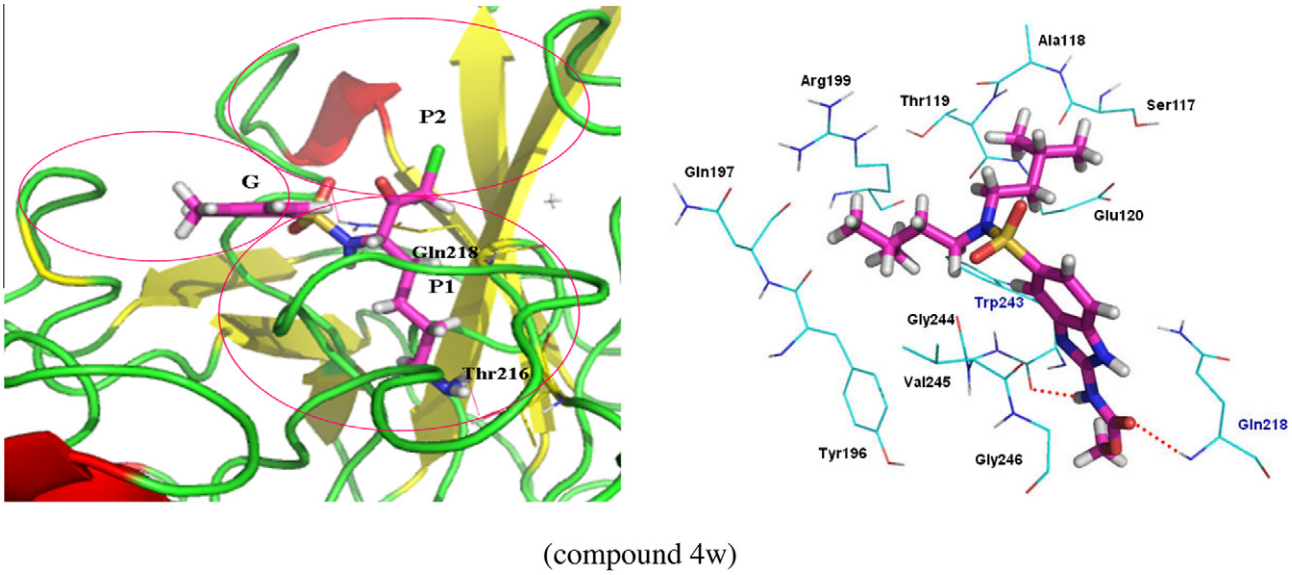


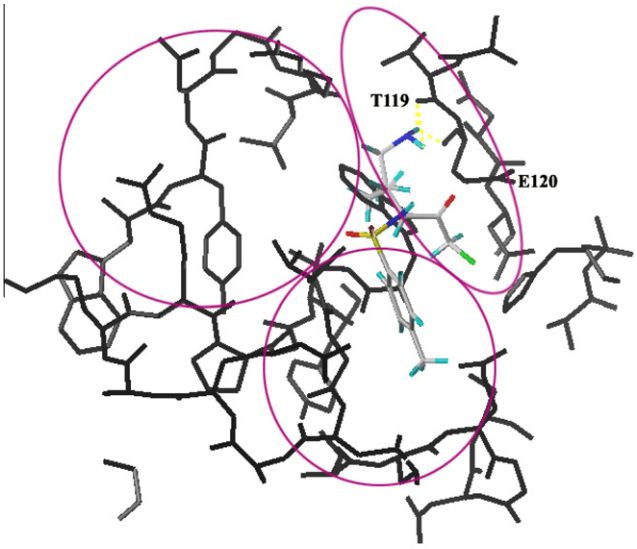
Figure 5. Identification of regions where acrosin protein interacts with various pharmacophores in compounds **4w** and compounds **4w** in the active site of acrosin.

compounds **4a**–**4s**. The structures of **4a**–**4s** were confirmed by ^{1}H NMR and mass spectral analysis.[19]

The in vitro acrosin inhibitory activities of the target compounds were evaluated by a modification of the method of Kennedy et al.,[20] and the results are listed in Table 1, in which TLCK was used as the control. The results showed that all title compounds had better acrosin inhibitory activities than that of the control TLCK.

In general, compounds with aliphatic chains (**4t**–**4x**) were more potent than compounds with aromatic rings (**4a**–**4s**). Compound **4w** containing isopentyl group displayed the most potent acrosin inhibitory activity among all the compounds, with an IC_{50} of 6.3×10^{-5} M. For the aromatic series, compounds with a halogen-substituted phenyl ring (**4b**–**4h**, **4n**–**4p**) were more active than compounds with a methyl or methoxy-substituted phenyl ring (**4i**–**4k**, **4q**–**4s**). In particular, compounds with a fluoro-substituted phenyl ring (**4b**–**4c**, **4n**–**4p**) showed better inhibitory activity than their chloro and bromo-substituted (**4d**–**4h**) analogs.

A molecular docking study was carried out using the Discovery Studio 2.5 software package.[21] The modes of action of compound **4w** and TLCK within the active site of human acrosin are shown in Figures 5 and 6. The results showed that TLCK is located in the P1 pocket and only forms hydrophobic interactions with hydrophobic residues lining the pocket (Fig. 6). As shown in Figures 4 and 5, the benzo[*d*]imidazole scaffold of target compound **4w** is located in the P1 and G pockets and forms hydrophobic interactions with the residues Trp243, Gly244, Val245 and Gly246. The carbamate group of compound **4w** is located in the P1 pocket and forms three pairs of hydrogen bonds with the key residues Gln218, Ser221 and Trp243. The two isoamyl groups of the sulfamide of compound **4w** extend into the P2 and G pockets, and form hydrophobic interactions with Tyr196, Gln197, Arg199, Gly244, Val245, Ser117, Ala118, Thr119, and Glu120. The results suggest that the hydrogen-bond interactions within the P1 pocket and the hydrophobic interactions within the P2 pocket can increase the acrosin inhibitory activities of target compounds.



(TLCK)

Figure 6. TLCK in the active site of acrosin.

In summary, a series of novel methyl 5-substituted 1*H*-benzo[*d*]imidazol-2-ylcarbamates were designed, synthesized and their acrosin inhibitory activities were evaluated in vitro. The results of acrosin inhibitory activity showed that all title compounds were more potent than that of the control TLCK. Compound 4w displayed the most potent acrosin inhibitory activity among all the compounds, with IC_{50} of 6.3×10^{-5} M, making it worth further study. The results identify a new structural class for the further development of novel acrosin inhibitory agents.

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References and notes

1. Brunnhuber, S.; Kirchengast, S. *Coll. Antropol.* **2002**, *26*, 467.
2. Coutinho, E. M.; Mascarenhas, I.; Acosta, O. M.; de Flores, J. G.; Gu, Z. P.; Ladipo, O. A.; Adekunle, A. O.; Otolorin, E. O.; Shaaban, M. M.; Abul Oyoon, M. *Clin. Pharmacol. Ther.* **1993**, *54*, 540.
3. Dickey, R. P. *Contraception* **2002**, *65*, 447.
4. Trlin, A. D.; Perry, P. E. *N. Z. Med. J.* **1982**, *95*, 700.
5. Rosatti, M. I.; Beconi, M. T.; Cordoba, M. *Biocell* **2004**, *28*, 311.
6. Cui, Y. H.; Zhao, R. L.; Wang, Q.; Zhang, Z. Y. *Asian J. Androl.* **2000**, *2*, 229.
7. Dejonge, C. J.; Tarchala, S. M.; Rawlins, R. J.; Radwanska, E. *Hum. Reprod.* **1993**, *8*, 253.
8. Pakzad, R. *Z. Mikrosk. Anat. Forsch.* **1989**, *103*, 8.
9. Jones, R.; Parry, R.; Lo Leggio; Nickel, P. *Mol. Hum. Reprod.* **1996**, *2*, 597.
10. Gupta, G.; Jain, R. K.; Maikhuri, J. P.; Shukla, P. K.; Kumar, M.; Roy, A. K.; Patra, A.; Singh, V.; Batra, S. *Hum. Reprod.* **2005**, *20*, 2301.
11. Bourinbaiar, A. S.; Lee-Huang, S. *Contraception* **1995**, *51*, 319.
12. Caflisch, A.; Miranker, A.; Karplus, M. *J. Med. Chem.* **1993**, *36*, 2142.
13. Zhang, J.; Zheng, C. H.; Sheng, C. Q.; Zhou, Y. J.; Zhu, J.; Lv, J. G. *Chem. J. Chin. Univ.* **2009**, *30*, 2409.
14. Jiang, J. H.; Liu, X. F.; Zheng, C. H.; Zhou, Y. J.; Zhu, J.; Lv, J. G.; Sheng, C. Q. *Chin. Chem. Lett.* **2011**, *22*, 272.
15. Liu, X. F.; Sheng, C. Q.; Lv, J. G., et al *J. Comput. Aided Mol. Des.* **2011**, *25*, 977.
16. Qi, J. J.; Zhu, J.; Liu, X. F.; Ding, L. L.; Zheng, C. H.; Han, G. Q.; Lv, J. G.; Zhou, Y. J. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 5822.
17. Morris, D. L.; Dykes, P. W.; Dickson, B., et al *Brit. Med. J.* **1983**, *236*, 103.
18. Lv, J. G.; Sheng, C. Q.; Zhang, M.; Ji, H. T.; Zhang, W. N.; Zhou, Y. J.; Zhu, J.; Jiang, J. H. *Acta Chim. Sinica* **2006**, *64*, 1073.
19. *Stuctural data for compounds* ***4a–4x***: *Compound* ***4a***: White solid; mp: 277–279 °C; ^{1}H NMR (300 MHz, DMSO) δ 11.30-11.72 (br, 2H), 9.30 (s, 1H), 7.67 (s, 1H), 7.47 (d, 1H), 7.36 (dd, 1H), 6.93 (d, 1H), 6.85 (d, 2H), 3.76 (s, 3H), 2.13 (s, 3H), 1.80 (s, 3H); MS (ESI) *m*/*z*: 373.19 [M−H]$^+$. Anal. calcd for $C_{17}H_{18}N_4O_4S$: C, 54.52; H, 4.81; N, 14.97; O, 17.10; S, 8.55. Found: C, 54.47; H, 4.77; N, 14.89; O, 17.05; S, 8.41. *Compound* ***4b***: White solid; mp: 257–259 °C; ^{1}H NMR (300 MHz, DMSO) δ 11.23–11.86 (br, 2H), 8.00 (t, 1H), 7.85 (s, 1H), 7.52 (s, 2H), 7.33 (t, 1H), 7.18–7.29 (m, 1H), 7.08 (dd, 2H), 3.95 (d, 2H), 3.77 (s, 3H); MS (ESI) *m*/*z*: 377.25 [M−H]$^+$. Anal. calcd for $C_{16}H_{15}FN_4O_4S$: C, 50.76; H, 3.97; F, 5.02; N, 14.81; O, 16.92; S, 8.46. Found: C, 50.66; H, 3.81; F, 5.09; N, 14.75; O, 16.84; S, 8.33. *Compound* ***4c***: White solid; mp: 258–261 °C; ^{1}H NMR (300 MHz, DMSO) δ 11.51–11.71 (br, 2H), 7.99 (t, 1H), 7.84 (s, 1H), 7.53 (s, 2H), 7.25 (dd, 2H), 7.07 (t, 2H), 3.90 (d, 2H), 3.77 (s, 3H); MS(ESI) *m*/*z*: 377.55 [M−H]$^+$. Anal. calcd for $C_{16}H_{15}FN_4O_4S$: C,50.72; H, 3.96; F, 5.02; N, 14.79; O, 16.91; S, 8.45. Found: C, 50.65; H, 3.88; F, 5.01; N, 14.63; O, 16.82; S, 8.33. *Compound* ***4d***: White solid; mp: 251–253 °C; ^{1}H NMR (300 MHz, DMSO) δ 11.50–12.30 (br, 2H), 8.04 (t, 1H), 7.87 (s, 1H), 7.54 (s, 2H), 7.39 (dd, 2H), 7.26 (pd, 2H), 4.00 (d, 2H), 3.77 (s, 3H); MS (ESI) *m*/*z*: 440.03 [M+H]$^+$. Anal. calcd for $C_{16}H_{15}BrN_4O_4S$: C, 48.12; H, 3.59; Br, 20.03; N, 14.04; O, 16.04; S, 8.02. Found: C, 48.05; H, 3.42; Br, 20.11; N, 14.01; O, 16.02; S, 8.12. *Compound* ***4e***: White solid; mp: 262–264 °C; ^{1}H NMR (300 MHz, DMSO) δ 11.50–11.69 (br, 2H), 8.02 (t, 1H), 7.83 (s, 1H), 7.53 (d, 2H), 7.39–7.48 (m, 2H), 7.18 (d, 2H), 3.89 (d, 2H), 3.77 (s, 3H).MS (ESI) *m*/*z*: 393.42[M−H]$^+$. Anal. calcd for $C_{16}H_{15}N_4O_4SCl$: C, 48.68; H, 3.80; Cl, 9.0; N, 14.20; O, 16.23; S, 8.11. Found: C, 48.59; H, 3.66; Cl, 8.94; N, 14.12; O, 16.10; S, 8.01. *Compound* ***4f***: White solid; mp: 260–262 °C; ^{1}H NMR (300 MHz, DMSO) δ 10.93–12.58 (br, 2H), 8.04 (t, 1H), 7.83 (s, 1H), 7.53 (d, 2H), 7.30 (dd, 2H), 7.23 (dd, 2H), 3.91 (d, 2H), 3.80 (s, 3H).MS (ESI) *m*/*z*: 393.31 [M−H]$^+$. Anal. calcd for $C_{16}H_{15}N_4O_4SCl$: C, 48.69; H, 3.80; Cl, 9.0; N, 14.20; O, 16.23; S, 8.11. Found: C, 48.50; H, 3.62; Cl, 8.85; N, 14.14; O, 16.13; S, 8.02. *Compound* ***4g***: White solid; mp: 247–249 °C; ^{1}H NMR (300 MHz, DMSO) δ 11.27–12.88 (br, 2H), 8.07 (t, 1H), 7.84 (s, 1H), 7.53 (s, 2H), 7.50 (d, 1H), 7.42 (d, 1H), 7.34 (dd, 1H), 3.99 (d, 2H), 3.77 (s, 3H).MS (ESI) *m*/*z*: 429.72 [M+H]$^+$. Anal. calcd for $C_{16}H_{14}N_4O_4SCl_2$: C, 44.78; H, 3.27; Cl, 16.56; N, 13.06; O, 14.93; S, 7.46. Found: C, 44.64; H, 3.18; Cl, 16.46; N, 13.01; O, 14.73; S, 7.31. *Compound* ***4h***: White solid; mp: 243–245 °C; ^{1}H NMR (300 MHz, DMSO) δ 11.58–12.18 (br, 2H), 8.08 (s, 1H), 7.80 (s, 1H), 7.50 (s, 2H), 7.48 (s, 1H), 7.39 (d, 1H), 7.21 (dd, 1H), 3.95 (d, 2H), 3.77 (s, 3H); MS (ESI) *m*/*z*: 429.36 [M+H]$^+$. Anal. calcd for $C_{16}H_{14}N_4O_4SCl_2$: C, 44.82; H, 3.27; Cl, 16.57; N, 13.07; O, 14.94; S, 7.47. Found: C, 44.71; H, 3.15; Cl, 16.43; N, 13.02; O, 14.82; S, 7.33. *Compound* ***4i***: White solid; mp: 217–220 °C; ^{1}H NMR (300 MHz, DMSO) δ 11.92–12.17 (br, 2H), 7.84 (s, 1H), 7.52 (s, 2H), 7.12 (d, 2H), 6.80 (d, 2H), 3.83 (d, 2H), 3.77 (s, 3H), 3.68 (s, 3H); MS (ESI) *m*/*z*: 389.33 [M−H]$^+$. Anal. calcd for $C_{17}H_{18}N_4O_5S$: C, 52.26; H, 4.61; N, 14.35; O, 20.50; S, 8.20. Found: C, 52.12; H, 4.48; N, 14.14; O, 20.42; S, 8.17. *Compound* ***4j***: White solid; mp: 255–259 °C; ^{1}H NMR (300 MHz, DMSO) δ 11.23–12.67 (br, 2H), 7.91 (s, 1H), 7.85 (s, 1H), 7.52 (s, 2H), 7.08–7.18 (m, 1H), 6.99 (d, 3H), 3.87 (d, 2H), 3.77 (s, 3H), 2.16 (s, 3H); MS (ESI) *m*/*z*: 375.38 [M+H]$^+$. Anal. calcd for $C_{17}H_{18}N_4O_4S$: C, 54.49; H, 4.80; N, 14.96; O, 17.09; S, 8.55. Found: C, 54.32; H, 4.65; N, 14.75; O, 17.01; S, 8.50. *Compound* ***4k***: White solid; mp: 242–243 °C; ^{1}H NMR (300 MHz, DMSO) δ 11.32–12.46 (br, 2H), 7.89 (s, 1H), 7.84 (s, 1H), 7.53 (s, 2H), 7.09 (d, 2H), 7.05 (d, 2H), 3.86 (d, 2H), 3.77 (s, 3H), 2.22 (s, 3H); MS (ESI) *m*/*z*: 375.10 [M+H]$^+$. Anal. calcd for $C_{17}H_{18}N_4O_4S$: C, 54.53; H, 4.81; N, 14.97; O, 17.11; S, 8.55. Found: C, 54.44; H, 4.68; N, 14.75; O, 17.07; S, 8.47. *Compound* ***4l***: White solid; mp: 253–256 °C; ^{1}H NMR (300 MHz, DMSO) δ 11.28–12.58 (br, 2H), 7.97 (t, 1H), 7.86 (s, 1H), 7.54 (s, 2H), 7.17–7.30 (m, 5H), 3.91 (d, 2H), 3.77 (s, 3H); MS (ESI) *m*/*z*: 359.24 [M−H]$^+$. Anal. calcd for $C_{16}H_{15}N_4O_4S$: C, 53.30; H, 4.16; N, 15.55; O, 17.77; S, 8.88. Found: C, 53.12; H, 4.07; N, 15.42; O, 17.65; S, 8.77. *Compound* ***4m***: White solid; mp: 265–268 °C; ^{1}H NMR (300 MHz, DMSO) δ 11.35–12.56 (br, 2H), 8.12 (t, 1H), 7.82 (s, 1H), 7.58 (d, 2H), 7.52 (s, 2H), 7.46 (d, 2H), 4.02 (d, 2H), 3.78 (s, 3H); MS (ESI) *m*/*z*: 428.82 [M−H]$^+$. Anal. calcd for $C_{17}H_{15}F_3N_4O_4S$: C, 47.46; H, 3.49; N, 13.03; O, 14.89; S, 7.44, F, 13.26. Found: C, 47.33; H, 3.31; N, 13.01; O, 14.76; S, 7.41, F, 13.10. *Compound* ***4n***: White solid; mp: 246–249 °C; ^{1}H NMR (300 MHz, DMSO) δ 11.33–12.41 (br, 2H), 8.00 (t, 1H), 7.83 (s, 1H), 7.51 (s, 2H), 7.35 (dd, 1H), 7.16–7.05 (m, 1H), 6.98 (dd, 1H), 3.93 (d, 2H), 3.77 (s, 3H); MS (ESI) *m*/*z*: 395.04 [M−H]$^+$. Anal. calcd for $C_{16}H_{14}F_2N_4O_4S$: C, 48.48; H, 3.53; N, 14.14; O, 16.16; S, 8.08; F, 9.60. Found: C, 48.23; H, 3.35; N, 14.01; O, 16.02; S, 8.05, F, 9.47. *Compound* ***4o***: White solid; mp: 249–252 °C; ^{1}H NMR (300 MHz, DMSO) δ 11.36–12.55 (br, 2H), 8.08 (t, 1H), 7.79 (d, 1H), 7.52 (s, 2H), 7.12–7.41 (m, 2H), 7.07 (s, 1H), 3.93 (d, 2H), 3.80 (s, 3H); MS (ESI) *m*/*z*: 395.57 [M−H]$^+$. Anal. calcd for $C_{16}H_{14}F_2N_4O_4S$: C, 48.42; H, 3.53; N, 14.12; O, 16.14; S, 8.07, F, 9.58. Found: C, 48.31; H, 3.24; N, 14.05; O, 16.24; S, 8.01, F, 9.42. *Compound* ***4p***: White solid; mp: 263–264°C; ^{1}H NMR (300 MHz, DMSO) δ 11.24–12.61(br, 2H), 8.04 (t, 1H), 7.85 (s, 1H), 7.53 (s, 2H), 7.29 (dd, 1H), 6.90–7.15 (m, 3H), 3.96 (d, 2H), 3.78 (s, 3H); MS (ESI) *m*/*z*: 377.57 [M−H]$^+$. Anal. calcd for $C_{16}H_{15}FN_4O_4S$: C, 50.72; H, 3.96; N, 14.79; O, 16.91; S, 8.45;F, 5.02. Found: C, 50.62; H, 3.73; N, 14.62; O, 16.86; S, 8.32, F, 5.12. *Compound* ***4q***: White solid; mp: 224–225°C; ^{1}H NMR (300 MHz, DMSO) δ 11.38–12.49 (br, 2H), 7.85 (s, 1H), 7.79 (t, 1H), 7.53 (s, 2H), 7.10–7.31 (m, 2H), 6.86 (t, 2H), 3.86 (d, 2H), 3.77 (s, 3H), 3.67 (s, 3H); MS (ESI) *m*/*z*: 389.57 [M−H]$^+$. Anal. calcd for $C_{17}H_{18}N_4O_5S$: C, 52.23; H, 4.61; N, 14.34; O, 20.48; S, 8.19. Found: C, 52.13; H, 4.55; N, 14.25; O, 20.32; S, 8.07. *Compound* ***4r***: White solid; mp: 217–219 °C; ^{1}H NMR (300 MHz, DMSO) δ 11.28–12.48 (br, 2H), 7.96 (t, 1H), 7.85 (s, 1H), 7.53 (s, 2H), 7.11–7.32 (m, 1H), 6.77 (dd, 3H), 3.89 (d, 2H), 3.77 (s, 3H), 3.65 (s, 3H); MS (ESI) *m*/*z*: 389.24 [M−H]$^+$. Anal. calcd for $C_{17}H_{18}N_4O_5S$: C, 52.28; H, 4.61; N, 14.35; O, 20.50; S, 8.20. Found: C, 52.14; H, 4.54; N, 14.22; O, 20.32; S, 8.11. *Compound* ***4s***: White solid; mp: 248–249 °C; ^{1}H NMR (300 MHz, DMSO) δ 10.89–12.50 (br, 2H), 7.85 (s, 2H), 7.51 (s, 2H), 6.79 (d, 1H), 6.71 (d, 2H), 3.86 (d, 2H), 3.78 (s, 3H), 3.67 (s, 3H), 3.60 (s, 3H); MS (ESI) *m*/*z*: 419.30 [M−H]$^+$. Anal. calcd for $C_{18}H_{20}N_4O_6S$: C, 51.39; H, 4.76; N, 13.32; O, 22.84; S, 7.61. Found: C, 51.14; H, 4.55; N, 13.25; O, 22.74; S, 7.52. *Compound* ***4t***: White solid; mp: 201–203 °C; ^{1}H NMR (300 MHz, DMSO) δ 11.26–12.57 (br, 2H), 7.86 (d, 1H), 7.54 (d, 2H), 7.42 (t, 1H), 3.77 (s, 3H), 3.03-3.22 (m, 1H), 0.90 (t, 6H); MS (ESI) *m*/*z*: 311.23[M−H]$^+$. Anal. calcd for

$C_{12}H_{16}N_4O_4S$: C, 46.12; H, 5.12; N, 17.94; O, 20.50; S, 10.25. Found: C, 46.05; H, 5.04; N, 17.74; O, 20.36; S, 10.11. *Compound* ***4u***: White solid; mp: 184–186 °C; ^{1}H NMR (300 MHz, DMSO) δ 10.70–12.65 (br, 2H), 7.83 (s, 1H), 7.44–7.63 (m, 2H), 7.35 (t, 1H), 3.74 (s, 3H), 2.67 (t, 2H), 1.30 (m, 2H), 1.19 (m, 2H), 0.76 (t, 3H); MS (ESI) *m*/*z*: 325.21 $[M-H]^+$. Anal. calcd for $C_{13}H_{18}N_4O_4S$: C, 47.82; H, 5.52; N, 17.17; O, 19.62; S, 9.81. Found: C, 47.65; H, 5.32; N, 17.09; O, 19.42; S, 9.66. *Compound* ***4v***: White solid; mp: 190–191 °C; ^{1}H NMR (300 MHz, DMSO) δ 11.08–12.39 (br), 7.82 (s), 7.46–7.61 (m, 2H), 7.36 (s, 1H), 3.77 (s, 3H), 2.67 (t, 2H), 1.17–1.32 (m, 2H), 1.02–1.17 (m, 6H), 0.77 (t, 3H); MS (ESI) *m*/*z*: 353.26 $[M-H]^+$. Anal. calcd for $C_{15}H_{22}N_4O_4S$: C, 50.81; H, 6.21; N, 15.80; O, 18.07; S, 9.03. Found: C, 50.69; H, 6.08; N, 15.57; O, 18.11; S, 9.14. *Compound* ***4w***: White solid; mp: 221–223 °C; ^{1}H NMR (300 MHz, DMSO) δ 11.37–12.45 (br, 2H), 7.80 (s, 1H), 7.51 (dd, 2H), 3.77 (s, 3H), 3.00 (t, 4H), 1.43–1.67 (m, 2H), 1.30 (q, 4H), 0.82 (d, 12H); MS (ESI) *m*/*z*: 409.42 $[M-H]^+$. Anal. calcd for $C_{19}H_{31}N_4O_4S$: C, 55.55; H, 7.55; N, 13.64; O, 15.59; S, 7.80. Found: C, 55.41; H, 7.39; N, 13.45; O, 15.23; S, 7.64. *Compound* ***4x***: White solid; mp: 267–269 °C; ^{1}H NMR (300 MHz, DMSO) δ 11.30–12.59 (br, 2H), 7.87 (s, 1H), 7.62 (d, 1H), 7.55 (dd, 1H), 7.16–7.46 (m, 5H), 4.07 (s, 2H), 3.78 (s, 3H), 3.31 (s, 3H); MS (ESI) *m*/*z*: 373.37 $[M-H]^+$. Anal. calcd for $C_{17}H_{18}N_4O_4S$: C, 54.49; H, 4.81; N, 14.96; O, 17.10; S, 8.55. Found: C, 54.30; H, 4.68; N, 14.65; O, 17.04; S, 8.32.

20. Kennedy, W. P.; Kaminski, J. N.; Vanderven, H. H.; Jeyendran, R. S.; Reid, D. S.; Blackwell, J.; Bielfeld, P.; Zaneveld, L. J. *J. Androl.* **1989**, *10*, 221.
21. Discovery Studio 2.5 Software Package AI, San Diego, CA (USA): http://www.accelrys.com.