Bioorganic & Medicinal Chemistry Letters 21 (2011) 5822–5825

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and acrosin inhibitory activities of substituted ethyl 5-(4-aminophenyl)-1*H*-pyrazole-3-carboxylate derivatives

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ARTICLE INFO

Article history:
Received 8 March 2011
Revised 11 July 2011
Accepted 28 July 2011
Available online 3 August 2011

Keywords:
Acrosin inhibitory agents
Synthesis
Ethyl 5-(4-aminophenyl)-1*H*-pyrazole-3-carboxylate

ABSTRACT

A series of novel ethyl 5-(4-aminophenyl)-1*H*-pyrazole-3-carboxylate derivatives were designed and synthesized and their in vitro acrosin inhibitory activities were evaluated. Most of the compounds exhibited acrosin inhibitory activities. Among them, three compounds (**5l**, **5n**, and **5v**) were more potent than that of the control TLCK. These provide a new structural type for the development of novel contraceptive acrosin inhibitory agents.

The problems of contraception are shared between men and women. Currently, modern contraceptive methods have protected millions of couples worldwide from unwanted pregnancy and allowed them to take control over their reproduction. However, forms of oral contraceptive pill. These can lead to serious side effects, such as most of these contraceptive methods are only available for women, especially the various breakthrough menstrual bleeding, nausea, mood swings, headaches, and an increased risk of rectal cancer.[1–4] Male contraception is largely limited to condoms and vasectomy. It is clear that there is a need for new contraceptive agents and methods which are easier to use with fewer side effects to place the burden of contraception more equitably between men and women.

Sperm acrosin is the major trypsin-like protease present in the acrosome of all mammalian spermatozoa and plays a critical role in fertilization.[5] Acrosin is a sperm-specific enzyme, possibly involved in the acrosome reaction, which functions in lysis of the zona pellucida and in facilitating penetration of the sperm through the innermost glycoprotein layers of the oocyte.[6] Insufficient levels of acrosin are associated with human infertility and inhibition of acrosin activity by protease inhibitors has been shown to reduce the success of fertilization.[7] Thus, it is a potential target for the design and development of novel male contraceptive agents. Since the function of acrosin was first reported, there has been much research on acrosin inhibitors as potential contraceptives. A number of small molecules acting as acrosin inhibitors, such as TLCK, AGB, DV-1006, and substituted isoxazolecarbaldehydes have been reported (Fig. 1).[8–10]

Our group has focused on a search for novel acrosin inhibitors for several years. In a previous study, our group constructed a homologous three-dimensional model of human acrosin based on the crystal structures of ram and boar form and the active site of human acrosin was analyzed using the multiple copy simultaneous search (MCSS) method.[11] Based on these studies, a potent acrosin inhibitory agent KF950 was designed and synthesized. This has been the subject of preclinical study.[12] Here, we report the synthesis and acrosin inhibitory activities in vitro of novel ethyl 5-(4-aminophenyl)-1*H*-pyrazole-3-carboxylate compounds.(Fig. 2; Table 1).

The chemical synthesis of ethyl 5-(4-aminophenyl)-1*H*-pyrazole-3-carboxylate derivatives is outlined in Scheme 1. The 1-(4-nitrophenyl)ethanone was oxalylated by diethyl oxalate in the presence of sodium ethanol, affording β-diketoester. The β-diketoester and hydrazine in refluxing ethanol yielded 5-(4-nitrophenyl)-1*H*-pyrazole-3-carboxylate (**3**). Then the reduction of (**3**) to the key intermediate ethyl 5-(4-aminophenyl)-1*H*-pyrazole-3-carboxylate (**4**). The target compounds **5a**–**5v** were synthesized by (**4**) with different acyl chlorides or anhydrides in the presence of triethylamine. The structures of these were determined by ^{1}H NMR and high resolution mass spectrum.

All the synthesized compounds were assessed for acrosin inhibitory activity using the method of Kennedy et al.[13] The activities of all the title compounds are listed in Table 1, in which TLCK was used

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doi:10.1016/j.bmcl.2011.07.110

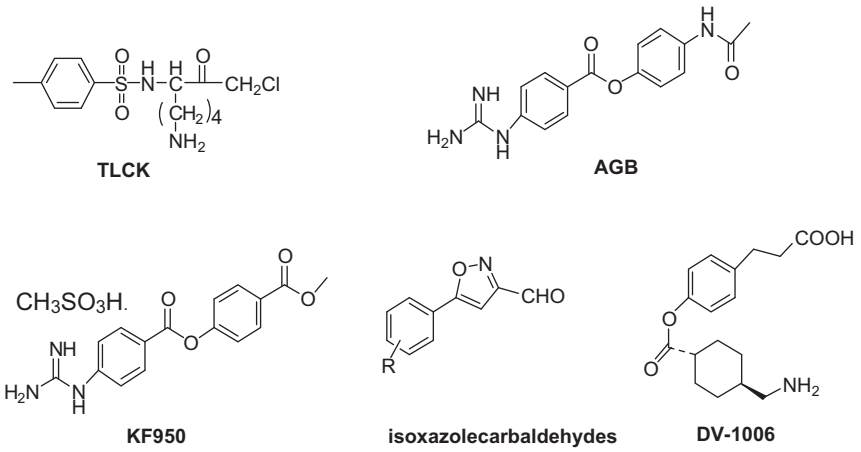


Figure 1. Chemical structures of TLCK, AGB, KF950, DV-1006, and substituted isoxazolecarbaldehydes.

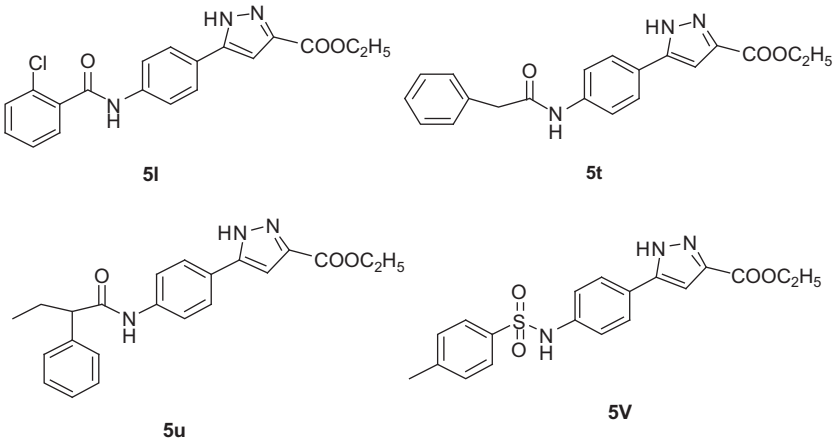


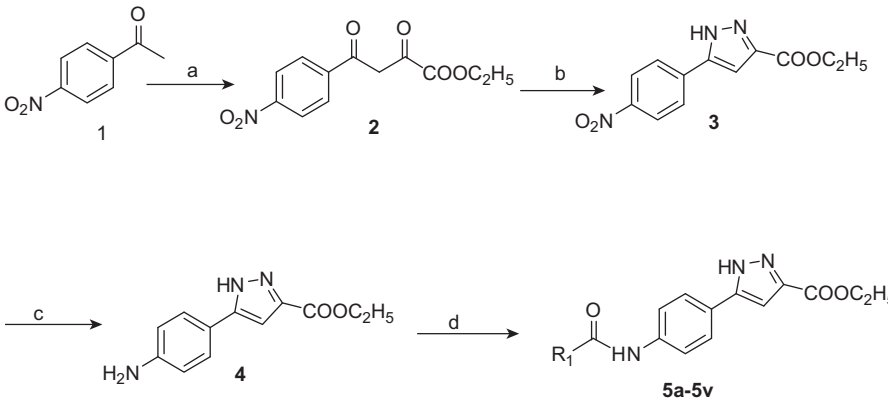
Figure 2. Chemical structures of the target compounds.

Table 1
Acrosin inhibitory properties of the target compounds

Compound	Acrosin inhibitor (IC_{50}) μmol/ml
5a	23
5b	14
5c	17
5d	72
5e	7.9
5f	5.76
5g	9.25
5h	17.4
5i	8.0
5j	2.4
5k	3.98
5l	0.11
5m	3.31
5n	0.44
5o	4.8
5p	2.4
5q	34.6
5r	0.8
5s	3.31
5t	0.47
5u	3.3
5v	0.032
TLCK	142.6

as the control. All of them showed better inhibitory activities than TLCK. Compounds belonging to aromatic series (**5i**–**5u**) were more potent than compounds belonging to aliphatic series (**5a**–**5h**). During the aromatic series, the compound bearing an unsubstituted phenyl ring (**5i**) was less active than the compounds having halogen substitutions (**5j**–**5p**). Halogen groups positioned at the para or ortho positions in the phenyl ring (**5j–5n**, **5p, 5s**) were the preferred substitutions for bioactivity. In contrast, compounds containing a chloro group on the phenyl ring (**5l**–**5n**) showed better acrosin inhibitory activities than their fluoro and bromo analogs. Derivatives with electron-donating substituents in the para position showed less acrosin inhibitory activity. Interestingly, compounds **5l** (with an *ortho*-chloro group on the phenyl ring) and **5n** (bearing two chlorine atoms at the 2- and 4-positions on the phenyl ring), exhibited much higher acrosin inhibitory activity than the TLCK control. In addition, compound **5v** containing a sulfonic group displayed very potent acrosin inhibitory activity with an IC_{50} of 0.032 μmol/ml.

The binding studies were carried out using Discovery Studio 2.5 software package (AI, San Diego, CA, USA). The modes of action of molecules **5l** and **5v** in biding with the active site of human acrosin are shown in Fig. 3. The 3-ethylester group of **5l** is located in the P1 pocket and its carbonyl oxygen forms a hydrogen bond with the key residue Ser221. The nitrogen of the pyrazole ring forms a hydrogen bond with the key residue Gln218. The ethyl 5-phenyl-1*H*-pyrazole-3-carboxylate group of **5v** is located in the P1 pocket and its carbonyl oxygen forms a hydrogen bond with the key residue Gln218. The imine group in the pyrazole ring forms a hydrogen-bonding interaction with the residue Val245. Moreover, the substituted benzene ring at the end extends to the P2 pocket,



5a R=CH_3
5b R=C_2H_5
5c R=C_3H_7
5d R=C_4H_9
5e R=CF_3
5f R=$(CH_2)_2Cl$
5g R=$(CH_2)_2COOH$
5h R=$(CH_2)_3COOH$

5i R=Ph
5j R=4-F-Ph
5k R=2,6-$(F)_2$-Ph
5l R=2-Cl-Ph
5m R=4-Cl-Ph
5n R=2,4-$(Cl)_2$-Ph
5o R=2,6-$(Cl)_2$-Ph

5p R=2-Br
5q R=4-CH_3-Ph
5r R=4-OCH_3-Ph
5s R=4-CH_2Cl-Ph
5t R=Ph-CH_2
5u R=C_2H_5CHPh
5v R=4-CH_3-$PhSO_2$

Scheme 1. Synthesis of the target compounds **5a**–**5v**. Reagents and conditions: (a) diethyl oxalate, C_2H_5ONa, C_2H_5OH, rt; (b) NH_2 $NH_2{\cdot}H_2O$, AcOH, reflux, 5 h; (c) 4 equiv $SnCl_2{\cdot}2H2O$, 6 equiv C_2H_5OH, $CH_3CO_2C_2H_5$, reflux, 6 h; (d) carbonyl chlorides, $(Et)_3N$, $CH_3CO_2C_2H_5$.

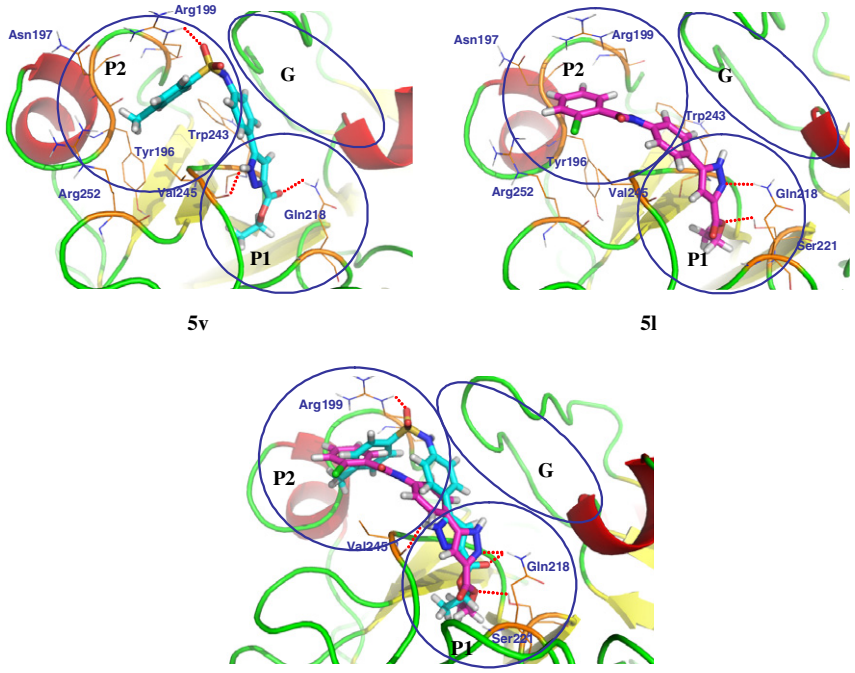


Figure 3. The mode of action of compounds **5v** and **5l**, superimposed on the active site of the homologous 3D model of human acrosin.

which is surrounded by hydrophobic residues lined with Tyr196, Asn197, Arg252, and Trp243. The oxygen in the sulfonamide group forms a hydrogen bond with the key residue Arg199, and this produced more potent activity than did compound **5l**. The docking model and the biological acrosin inhibitory tests provide ideas for further design and for synthesizing their derivatives.

In summary, a series of novel ethyl 5-(4-aminophenyl)-1*H*-pyrazole-3-carboxylate derivatives were designed and synthesized. An in vitro biological assay indicated that the target compounds had more potent acrosin inhibitory activity than the positive control compound TLCK. In particular, compounds **5l**, **5n**, and **5v** exhibited similar activity to KF950 and are worthy of further evaluation.

Acknowledgment

Financial support was provided from the Science and Technology Support Program, Jiang Su 2010, BE2010682.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.07.110.

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