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Synthesis and herbicidal evaluation of novel benzothiazole derivatives as potential inhibitors of D1 protease

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ABSTRACT

D1 protease is a C-terminal processing protease that has been predicted to be an ideal herbicidal target. Three novel series of benzothiazole derivatives were synthesized and evaluated for their herbicidal activities against *Brassica napus* (rape) and *Echinochloa crusgalli* (barnyard grass). The preliminary bioassay indicated that most of the synthesized compounds possess promising D1 protease inhibitory activities and considerable herbicidal activities. Molecular docking was performed to position representative compounds into the active site of D1 protease to determine a probable binding model.

The photosystem II D1 C-terminal processing protease (D1 protease, CtpA) is encoded by CtpA gene and responsible for cleaving D1 precursor protein to form mature D1 protein in the chloroplast of green plants.[1,2] This process is essential for the assembly of a manganese cluster and consequent light-mediated water oxidation in photosystem II, which is crucial for plant growth.[3,4] Diner and co-workers[5] reported that knockout of the CtpA gene results in a non-photoautotrophic phenotype due to an inability to form mature D1. Thus, the inhibitors of the D1 protease that target the photosynthetic apparatus of plants could constitute a broad new class of herbicides.[6] Moreover, D1 protease has high homology and lower abundance than D1 protein in the thylakoids of higher plants.[7] It is thus likely that much lower amounts of an effective D1 protease inhibitor would be required than the widely applied herbicides targeting D1 protein.[8] The frequent use of herbicides has resulted in many resistant weed species and no herbicide with a new molecular target sites has been commercialized in the last 20 years.[9,10] Identifying and synthesizing new compounds that target D1 protease in plants may be a valuable strategy for developing efficient and environmentally friendly herbicides.[11]

Although the X-ray crystal structure of the D1 protease was reported in 2000, relatively few small molecule inhibitors of D1 protease have been reported.[12] Duff et al.[6] described a screening assay that led to the discovery of five novel chemical classes of CtpA inhibitors. These lead compounds, including rhodanine dimers, ketoheterocycles, benzoxazinones, CF_3 peptides and imidazoles were evaluated for their inhibitory activities against both recombinant and native spinach CtpA. Zhang and co-workers[7] designed and synthesized a series of novel 4-(4-(5-methyl-3-arylisoxazol-4-yl)thiazol-2-yl)piperidyl carboxamides and thiocarboxamides as potential inhibitors targeting D1 protease in plants. In this Letter, we intend to develop novel and effective inhibitors against D1 protease.

In the work of Duff et al. described above, a chiral alpha-ketoheterocycle compound bearing a benzothiazole moiety (benzothiazole ketone), was identified as a potential inhibitor of D1 protease and was shown to possess significant herbicidal activity. However, it should be noted that only the racemate was evaluated and the single active enantiomer was not reported in the literature.[6] The active site of D1 protease consists of two hydrophobic pockets, which are formed by the residues Phe-140, Leu-152, Leu-212, Tyr-213, Val-324, Val-337, Ile-339, Ile-348, Tyr-349, Val-376, Ile-400, Val-403 and Tyr-419.[12] As shown in Figure 1, a proposed pharmacophore model consisting of a benzothiazole moiety and two suitably substituted phenyl rings bound to a linker was hypothesised on the basis of the crystal structure of D1 protease. To increase the structural diversity and discover more potent inhibitors of D1 protease, three novel benzothiazoles were designed and synthesized based on the structure of lead compound and topological regions of D1 protease (Fig. 1). Their herbicidal

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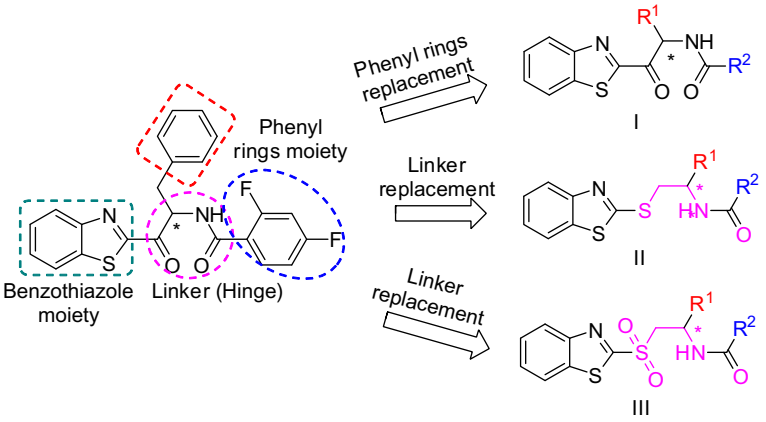
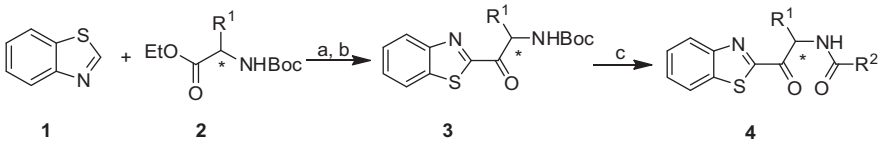
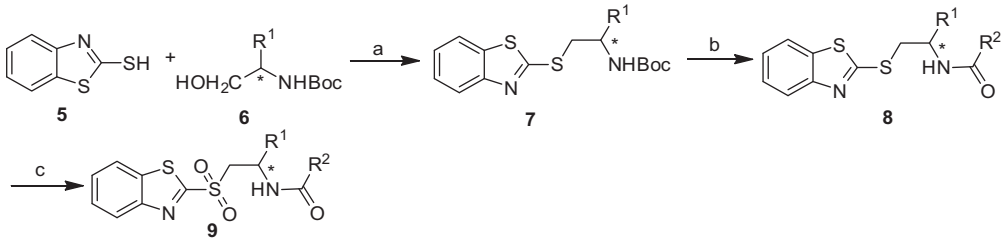


Figure 1. Design strategy of the target compounds.

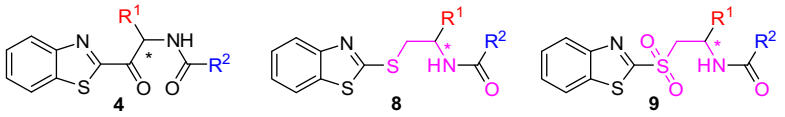


Scheme 1. Reagents and conditions: (a) *n*-BuLi, THF, −78 °C; (b) THF, 5–10 °C; (c) (i) TFA, DCM; (ii) carboxylic acid, HATU, TEA, DMF, rt.



Scheme 2. Reagents and conditions: (a) DEAD, PPh_3, THF, rt; (b) (i) TFA, CH_2Cl_2; (ii) carboxylic acid, HATU, TEA, DMF, rt; (c) *m*-CPBA, CH_2Cl_2, rt.

Table 1
Log*P* measurements, in vitro inhibitory activities of D1 protease and herbicidal activities (inhibition rate/%)



Compd	Config.	R^1	R^2	CtpA IC_{50} (μM)	Log*P*	Relative inhibition			
						Rape		Barnyard grass	
						100 mg/L	10 mg/L	100 mg/L	10 mg/L
4a	*R*	Me	*t*-BuO	>50	4.58	39	21	41	17
4b	*S*	Me	*t*-BuO	>50	4.58	38	17	34	9
4c	*R*	Bz	*t*-BuO	>50	4.69	50	24	48	24
4d	S	Bz	*t*-BuO	>50	4.69	43	31	43	12
4e	*R*	Bz	4-Fluorophenyl	29.4	4.40	61	34	53	30
4f	*S*	Bz	4-Fluorophenyl	44.3	4.40	50	28	42	29
4g	*R*	Bz	2,4-Difluorophenyl	3.2	4.49	79	59	68	52
4h	*S*	Bz	2,4-Difluorophenyl	36.3	4.49	62	42	47	37
4i	*R*	Bz	3,4-Dimethoxyphenyl	24.9	4.82	75	66	69	43
4j	S	Bz	3,4-Dimethoxyphenyl	41.3	4.82	72	55	57	26
4k	*R*	Bz	(2,4-Dichlorophenoxy)methyl	2.5	3.85	97	95	46	20
4l	*S*	Bz	(2,4-Dichlorophenoxy)methyl	10.4	3.85	95	83	50	32
8a	*R*	Ph	*t*-BuO	>50	5.48	48	24	49	11
8b	*S*	Ph	*t*-BuO	>50	5.48	37	20	41	0
8c	*R*	Bz	*t*-BuO	>50	5.69	61	29	49	20

(*continued on next page*)

Table 1 (*continued*)

Compd	Config.	R^1	R^2	CtpA IC_{50} (μM)	Log*P*	Relative inhibition			
						Rape		Barnyard grass	
						100 mg/L	10 mg/L	100 mg/L	10 mg/L
8d	*S*	Bz	*t*-BuO	>50	5.69	48	25	51	6
8e	*R*	Bz	4-Fluorophenyl	>50	5.85	66	22	47	18
8f	*S*	Bz	4-Fluorophenyl	>50	5.85	43	28	33	19
8g	*R*	Bz	2,4-Difluorophenyl	32.5	5.94	62	41	29	27
8h	*S*	Bz	2,4-Difluorophenyl	48.6	5.94	50	43	34	21
9a	*R*	Ph	*t*-BuO	>50	4.36	53	33	48	36
9b	*S*	Ph	*t*-BuO	>50	4.36	44	21	46	31
9c	*R*	Bz	*t*-BuO	>50	4.57	61	38	63	40
9d	S	Bz	*t*-BuO	>50	4.57	50	33	60	38
9e	*R*	Bz	4-Fluorophenyl	34.6	4.73	78	53	39	26
9f	*S*	Bz	4-Fluorophenyl	46.1	4.73	72	45	50	24
9g	*R*	Bz	2,4-Difluorophenyl	14.5	4.82	87	74	47	38
9h	*S*	Bz	2,4-Difluorophenyl	31.4	4.82	90	70	44	39
4m	*Racemate*	Bz	2,4-Difluorophenyl	28.5	4.49	74	42	53	46
Atrazine				>50	2.55	95	86	85	76

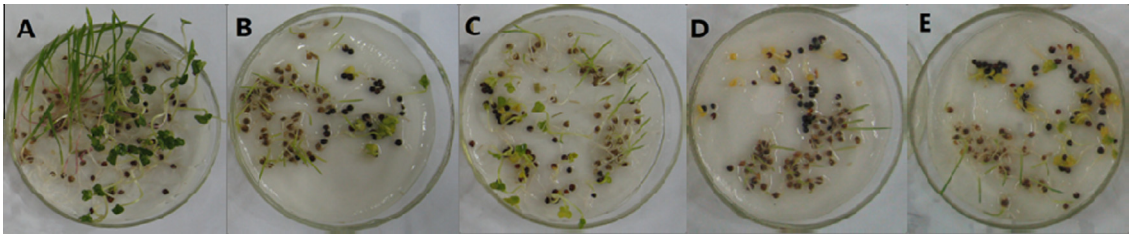


Figure 2. Comparison of the effects of compound **4k** and Atrazine on plants growth. (A) Wild-type rape and barnyard grass grown on regular medium. All plants grow normally. (B) Wild-type rape and barnyard grass grown on medium containing 100 ppm of compound **4k**. Most plants show stunted growth or chlorotic phenotype. (C) Compound **4k** (10 ppm). (D) Atrazine (100 ppm). (E) Atrazine (10 ppm).

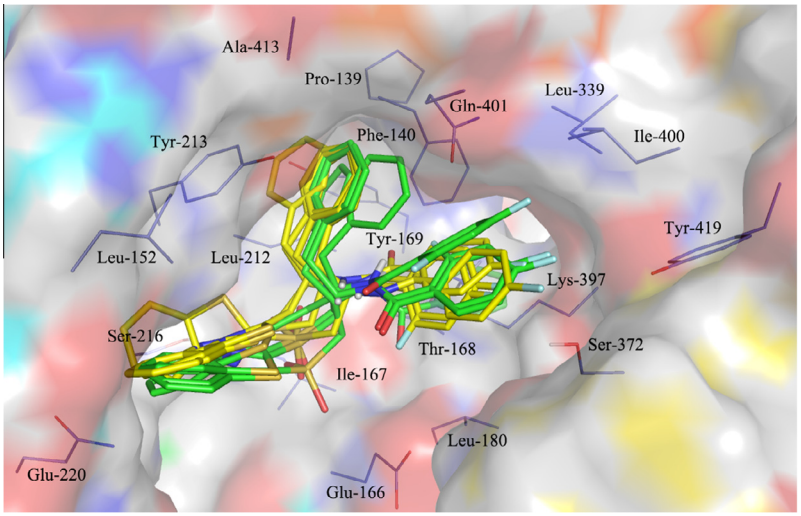


Figure 3. Overlay of D1 protease with compounds of **4e**, **4g**, **8e**, **9e**, **9g** (yellow), **4f**, **8h**, **9f**, **9h** (green). Molecular surface of the binding site with rainbow colors formed by residues Phe-140, Leu-152, Leu-212, Tyr-213, Leu-399, Ile-400, and Tyr-419.

activities against *Brassica napus* (rape) and *Echinochloa crusgalli* (barnyard grass) were evaluated. Preliminary HPLC enzyme assays[7] were also performed to evaluate the native spinach D1 protease inhibitory activities. Additionally, molecular docking was carried out to investigate the possible binding mode of typical compounds with D1 protease.[13,14]

The general synthesis of substituted benzothiazole ketones, benzothiazole sulfides and benzothiazole sulfones are outlined in Schemes 1 and 2. As shown in Scheme 1, commercially available benzothiazole **1** was converted to benzothiazol-2-yllithium in the presence of *n*-BuLi at low temperature. Benzothiazol-2-yllithium was not isolated and directly reacted with single enantiomers of Boc-Glycine ethyl ester derivatives **2** to afford the intermediates **3**. Boc group on the intermediates **3** were removed with trifluoroacetic acid (TFA) in dichloromethane (DCM) at room temperature, followed by amidation in the presence of 1-

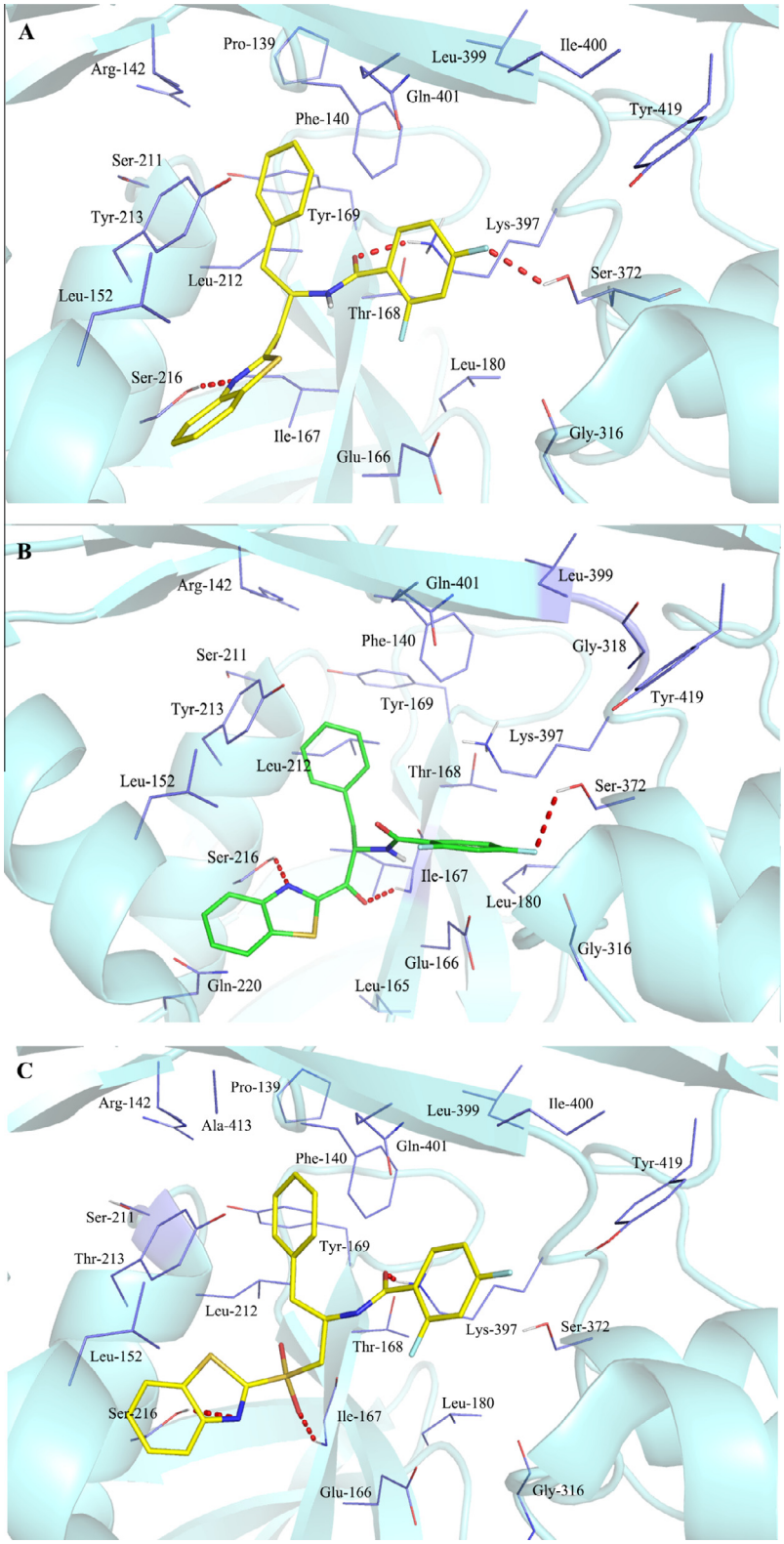


Figure 4. (A) Molecular docking model for compound **4g** (yellow and stick) with the active site of D1 protein, highlighting the hydrogen bonds (red dashed lines) coordination between the compound **4g** and amino acid residues Ser-216, Lys-397 and Ser-372. (B) Molecular docking model for compound **4h** (green and stick) with the active site of D1 protein, highlighting the hydrogen bonds (red dashed lines) coordination between the compound **4h** and the amino acid residues Ser-216, Ile-167 and Ser-372. (C) Molecular docking model for compound **9g** (yellow and stick) with the bonding pocket of D1protein, the hydrogen bond (red dashed lines) were formed between the compound **9g** and amino acid residues Ser-216, Ile-167 and Lys-397.

[Bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate (HATU) and triethylamine (TEA) to afford the desired substituted benzothiazole ketone derivatives **4**.

As shown in Scheme 2, the intermediates **7** were obtained by the reaction of commercially available benzothiazolethiol **5** with single enantiomers of Boc-glycinol derivatives **6** in the presence of diethyl azodicarboxylate (DEAD) and triarylphosphines (PPh_3) at room temperature. The intermediates **7** further underwent *N*-Boc deprotection and amidation to give intermediate benzothiazole sulfide derivatives **8**. The resulted intermediates **8** were then reacted with *m*-chloroperoxybenzoic acid (*m*-CPBA) in DCM at room temperature to afford the corresponding benzothiazole sulfone derivatives **9**.

All the synthesized compounds **4a**–**4m**, **8a**–**8h** and **9a**–**9h** were evaluated for herbicidal activities against barnyard grass and rape at dosages of 100 mg/L and 10 mg/L according to a previously reported procedure.[15,16] Atrazine, a commercial herbicide, was used as a control. The parent compound **4m** was used as another control. The in vitro inhibitory activities of these compounds were further evaluated against the native spinach D1 protease. The octanol–water partition coefficient log*P* for these compounds was calculated using the online Molinspiration log*P* calculator.[17] For the convenience of structure–activity relationship analysis, compounds **4a**–**4m**, **8a**–**8h** and **9a**–**9h** were defined as benzothiazole ketone derivatives, benzothiazole sulfide derivatives and benzothiazole sulfone derivatives, respectively. The bioassay results (Table 1) indicate that most of the synthesized compounds showed moderate to good herbicidal activities inhibiting the growth of barnyard grass and rape at a dosage of 100 mg/L. For instance, **4k**, **4l**, **9g** and **9h** exhibited inhibitory rates of >80% to the growth of rape at a concentration of 100 mg/L. However, when the concentration was decreased to 10 mg/L, the inhibitory activities of **9g** and **9h** decreased obviously in comparison with the commercial herbicide Atrazine. In general, the herbicidal activities of *R* enantiomers were higher than their corresponding *S* enantiomers. The herbicidal activities of benzothiazole sulfone derivatives are higher than the corresponding benzothiazole sulfide derivatives, which have higher log*P* values. This implies that the lipophilicity may contribute to the variation. For example, the benzothiazole sulfone derivative **9g** (R^1 = benzyl, R^2 = 2,4-difluorophenyl; 87; 74; 47; 38; log*P* = 4.82) displayed higher herbicidal activities than the corresponding benzothiazole sulfide derivative **8g** (R^1 = benzyl, R^2 = 2,4-difluorophenyl; 62; 41; 29; 27; log*P* = 5.94). In addition, **4i**, **4j**, **4k**, **4l**, **9e**, **9f**, **9g** and **9h** showed stronger inhibition of the growth of the dicotyledon rape than that of the monocotyledon barnyard grass and exhibited a relative selectivity at the same concentration.

The in vitro inhibitory activities of these compounds against native spinach D1 protease were obtained with an HPLC assay method[7] by employing a mimic polypeptide substrate 24-mer oligopeptide (S24). From the data in Table 1, we can conclude that the sequence of inhibitory activities against D1 protease is benzothiazole ketone derivatives > benzothiazole sulfone derivatives > benzothiazole sulfide derivatives. For example, the benzothiazole ketone derivative **4g** (R^1 = Bz, R^2 = 2,4-difluorophenyl, *R* enantiomer) displayed better D1 protease inhibitory activity than the corresponding benzothiazole sulfone derivative **9g**, while the benzothiazole sulfide derivative **8g** showed the least inhibitory activity with IC_{50} value of 32.5 μM. In addition, the *R*-enantiomer was found to have more potent D1 protease inhibitory activities as compared to its corresponding *S*-enantiomer and racemate. For example, compound **4g** (*R* enantiomer) showed a better D1 protease inhibitory activity with an IC_{50} value of 3.2 μM than its corresponding *S* enantiomer **4h** (IC_{50} value 36.3 μM) and racemate **4m** (IC_{50} value 28.5 μM). Among these three series of compounds, replacing the R^2 group with *t*-BuO group reduced the inhibition of D1 protease. For example, **4a**–**4d**, **8a**–**8d**, **9a**–**9d** showed D1 protease inhibitory activities with IC_{50} values more than 50 μM.

The growth inhibition phenotype of typical compound **4k** on rape (*Brassica campestris* L.) and barnyard grass (*Echinochloa crus-galli* L.) at dosages of 10 mg/L and 100 mg/L is shown in Figure 2B and C. The controls obtained from the inhibition by Atrazine (Fig. 2D and E) and blank (Fig. 2A) are also shown for comparison. Obviously, both the compound **4k** and Atrazine exhibit phytotoxicity against rape and barnyard grass either at the dosage of 100 mg/L or 10 mg/L. The stunted growth or chlorotic phenotype (Fig. 2B and C) may be attributed to the inhibition of D1 protease and affected the functions of photosystem II in test plants.

D1 protease has a high homology in organisms and the crystal structure of D1 protease of higher plants has not been reported. To investigate the interactions of the benzothiazole derivatives with the active site of D1 protease, the three-dimensional structure of D1 protease of spinach was homology modeled based on the crystal structures of D1 protease from green alga *Scenedesmus obliquus* (PDB: 1FC6).[12] The synthesized compounds were energy minimized and docked into the active site of D1 protease, which is formed by residues of Phe-140, Leu-152, Leu-212, Tyr-213, Leu-339, Leu-399, Ile-400 and Tyr-419. The docked representative compounds **4e**, **4f**, **4g**, **4e**, **4h**, **9e**, **9f**, **9g**, **9h** clustered in the T shaped active site (Fig. 3). These compounds are shaped like a clover leaf and exhibited similar conformations and binding modes. The benzene rings of the compounds are stabilized by π–π interactions with Tyr-213 in the binding pocket. A hydrogen bond is formed between the N atom of benzothiazole moiety of the typical compounds and the side chain amide of Ser-216, thus binding the benzothiazole moiety in a hydrophobic pocket. The molecular docking model for compounds **4g** and **4h** may explain the molecular basis of stereoselectivity. Compound **4g** has a higher docking score and superior inhibitory activity in comparison to **4h**, which is to a certain extent consistent with the in vivo herbicidal activities. As shown in Figure 4A and B, the *R* stereoisomer of **4g** forms interactions between the carbonyl group and the critical amino acid residue Lys-397, while the orientation of the carboxyl of the *S* stereoisomer **4h** was opposite to that of Lys-397 resulting in a decreased binding energy and a lower docking score. In addition, a hydrogen-bond interaction between the benzothiazole sulfone derivatives **9g** and the side chain of Ser-372 is missing in the Figure 4C, we speculate that the missing interaction may contribute to the lower activity of **9g** than compound **4g**.

In conclusion, three new series of benzothiazole derivatives were synthesized and evaluated for their inhibitory activities against native spinach D1 protease and herbicidal activities against barnyard grass and rape. The preliminary enzyme assay results indicated that the *R* enantiomers possess better D1 protease inhibitory activities than the *S* enantiomers. Most of the synthesized compounds showed moderate to good herbicidal activities at dosages of 100 mg/L and 10 mg/L. Molecular docking revealed the probable binding model and molecular basis of binding stereoselectivity. Further investigation on the structural optimization of these compounds to improve the herbicidal activities and the in vitro activities against D1 protease is still in progress in our laboratory.

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Supplementary data

Supplementary data (synthetic procedure and characterization for all the new compounds, general procedures for the biological activity) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.01.087.

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