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Exploration of Novel Ureidobenzothiazole Library Against Neuroinflammation

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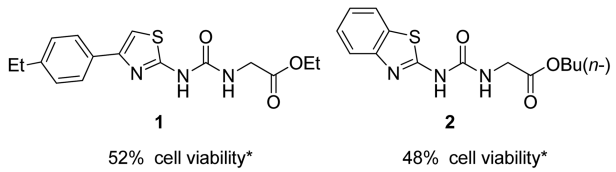
Combinatorial chemistry serves as a powerful tool in lead discovery and lead optimization by facilitating the rapid generation of potential candidates for screening. Lead optimization involves structural modifications of a hit compound to a lead compound that has demonstrated the desired biological or pharmacological activities, often in an in-vitro assay system. Among the broad range of templates, heterocyclic scaffolds represent the most promising molecules as leading structures for the discovery of novel synthetic drugs. In particular, the 2-aminothiazole core is found in numerous drugs and clinical and preclinical candidates that address a broad spectrum of targets.[1] For example, the 2-aminothiazole compounds bind to enzymes such as cyclooxygenases,[2] phosphodiesterases,[3] kinases[4] and acetylcholinesterase[5] and thereby attach to receptors such as integrins and various members of the GPCR family. Recently, the 2-aminothiazole-bearing compounds were reported as a new class of small molecules with antiprion activity in prion-infected neuroblastoma cell lines,[6] and as lead compounds multitargeted against Alzheimer disease and anticholinesterase as a single chemical entity in a more balanced biological profile.[7]

As a part of our efforts to search for a new compound that is useful for treating neurodegenerative disorders, we have been examining a limited set of selected compounds from our in-house indigenous chemical library. From the primary screening we identified two compounds **1** and **2** which showed a protective effect against neuroinflammation in β-amyloid induced neurotoxicity in PC12 cells and cortical and mesencephalic neurons (Chart 1).[8]

With these initial data, we decided to synthesize analogues of compounds **1** and **2** for hit-to-lead optimization. Considering the structures and activities of compounds **1** and **2**, we speculated that the 1,3-thiazole core (I) and urea (II) moiety would play a critical role in their biological activities (Chart 2). Therefore, we elaborated a synthetic strategy for preparing the compounds bearing three diversity points (I-III) in one molecule. Numerous analogues of the libraries were assumed to be readily prepared through coupling reactions between the primary amine and isocyanate or isothiocyanate with a suitable building block collection (see Scheme 1 for retrosynthetic analysis). Additionally, the ester moiety would allow the diversity of the library to afford an additional sublibrary. Another point to be considered in our synthetic strategy was a spacer between the nitrogen atom in



*% cell viability: determined by lactate dehydrogenase (LDH) assay kit at a concentration of 50 μM.[8]

Chart 1. Hit compounds explored from primary screening.

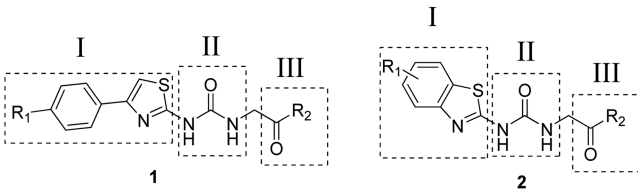
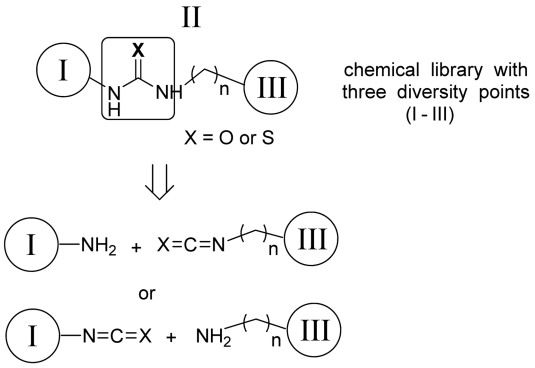


Chart 2. Fragment analyses of structures **1** and **2**.



Scheme 1. Retrosynthetic analysis of chemical library with three diversity points.

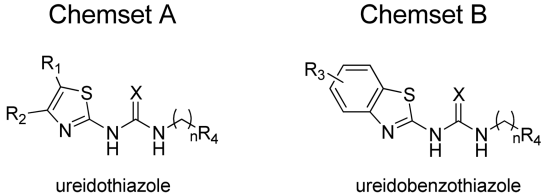
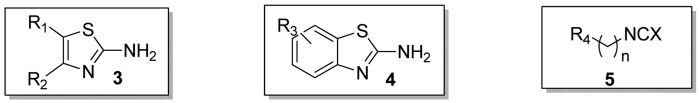


Chart 3. Chemical library of the two categories targeted in the synthesis.



3a : R_1= H, R_2= C_6H_4(4-Cl)
3b : R_1= H, R_2= C_6H_4(4-CH_3)
3c : R_1= H, R_2= C_6H_4(4-Br)
3d : R_1= H, R_2= C_6H_4(4-*n*-Bu)
3e : R_1= H, R_2= C_6H_4(4-Et)
3f : R_1= H, R_2= H
3g : R_1= CH_3, R_2= H
3h : R_1= H, R_2= CH_3

4a : R_3= 6-CH_3
4b : R_3= 6-F
4c : R_3= 4-Cl
4d : R_3= 6-NO_2
4e : R_3= 6-OCH_3
4f : R_3= 6-OCH_2CH_3
4g : R_3= H
4h : R_3= 5,6-di CH_3
4i : R_3= 6-Cl
4j : R_3= 4-CH_3
4k : R_3= 4-OCH_3

5a : R_4=CO_2Et, n=1, X=O
5b : R_4=CO_2Et, n=2, X=O
5c : R_4=CO_2Et, n=3, X=O
5d : R_4=CO_2(*n*-Bu), n=1, X=S
5e : R_4=CO_2Et, n=0, X=S
5f : R_4=CO_2Et, n=1, X=S
5g : R_4=C_6H_4(4-OCH_3), n=0, X=O
5h : R_4=C_6H_4(4-CH_3), n=1, X=O

Figure 1. Experimental building blocks 2-amino-1,3-thiazole **3a-3h**, 2-amino-1,3-benzothiazole **4a-4k** and isocyanate and isothiocyanate **5a-5h**.

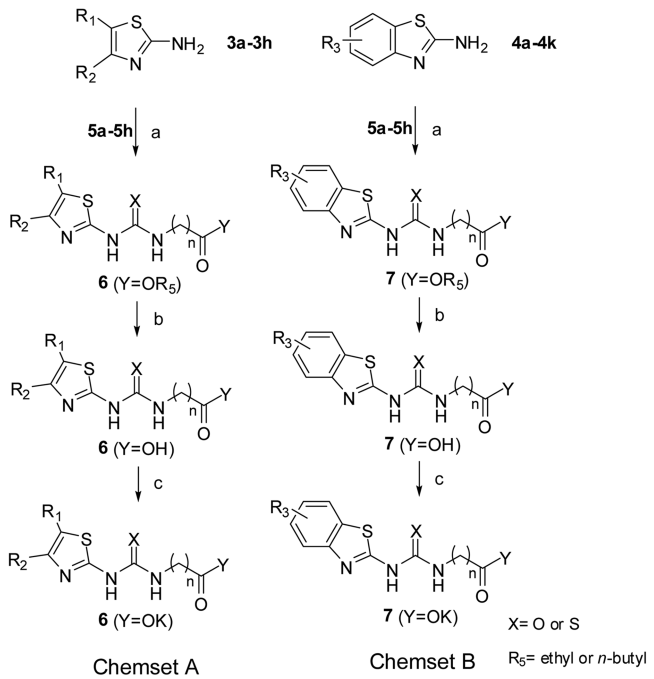
urea (or thiourea) and III.

The chemical library for exploring the candidate was constructed through two categories (Chemsets A and B in Chart 3).

The building blocks 2-amino-1,3-thiazole **3** and 2-amino-1,3-benzothiazole **4** were easily prepared by the reaction of the commercially available corresponding thiourea with α-haloketone in refluxing ethanol in quantitative yields.[9] The prepared compounds **3a-3h** and **4a-4k** and the other building blocks used in the study, isocyanates and isothiocyanates **5a-5h**, are listed in Figure 1, respectively.

A synthetic route to ureido-1,3-thiazoles and ureido-benzo-1,3-thiazoles by a parallel combinatorial method is shown in Scheme 2.

The reaction of the prepared building block **3a-3h** with **5a-5f** in anhydrous boiling tetrahydrofuran gave the corresponding ureido-1,3-thiazoles **6**. The reaction proceeded quantitatively and the products were obtained as a solid. The desired products, **6** could be isolated simply by filtration from the reaction mixture after the completion of reaction, with isolated yields ranging from 11 to 87%. The products **6** were confirmed as a single compound respectively by TLC and their ^{1}H NMR spectra agreed with the corresponding structures. Under the same aforementioned reaction conditions, the ureido-benzo-1,3-thiazoles **7** were obtained by the reaction of **4a-4k** with the building block **5a-5h**, with isolated yields ranging from 6-89%. We constructed libraries of 12 and 29 compounds of Chemsets A and B, respectively, in this manner using Carousel Reaction Stations in a parallel synthetic fashion.



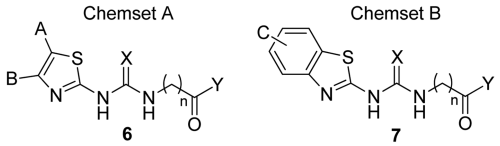
Reagent and conditions: (a) THF, reflux; (b) KOH (3.0-4.0eq), MeOH/H_2O, reflux and then conc. HCl, rt; (c) KOH (1.0eq), H_2O, rt.

Scheme 2. Overall synthetic routes of Chemsets A and B.

The ethyl ester function in molecules **6** and **7** was further transformed to the corresponding acids **6** (Y=OH) and **7** (Y=OH) through hydrolysis by treatment of aqueous potassium hydroxide solution at reflux temperature in 24-91% yields. The product was confirmed by the disappearance of the quartet at 4.17 ppm (J = 7.0 Hz) and the triplet at 1.19 ppm (J = 7.0 Hz), which corresponds to the ethyl protons in their ^{1}H NMR spectra. To enhance the solubility of the acids **6** (Y=OH) and **7** (Y=OH) in water, they were converted to the corresponding potassium salt **6** (Y=OK) and **7** (Y=OK), respectively. We constructed libraries of 8 and 18 compounds of Chemsets A and B, respectively, in this manner.

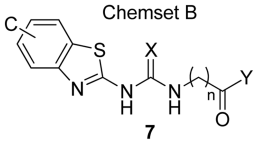
Primary screening results of the synthesized compounds at a concentration of 50 μM are partially summarized in Table 1. This result suggested that the Chemset B (compounds 7 series) is likely in the activity. Therefore, we constructed a focused library of benzothiazole derivatives and their primary screening results at a concentration of 500 nM are partially summarized in Table 2 (See the Supporting Information for the screening results of the synthesized compounds). The structure-activity relationship depending on the substituents (R_1, R_2 and R_3) of the aminothiazole scaffolds could not be precisely determined due to lack of ureidothiazole and ureidobenzothiazole derivatives, but the spacer from the carbonyl group to the nitrogen atom in urea (or thiourea) in the compound seemed to exert an important role in the activity. Further studies on expanding the library of Chemsets A and B and on elucidating the structure activity relationship of these series are in progress.

Table 1. Partial primary screening results of the synthesized compounds at a concentration of 50 μM



Entry	Compound	A	B	C	X	Y	n	% cell viability (at 50 μM)
1	**6a**	H	C_6H_4(4-Cl)	-	O	OEt	1	68
2	**6b**	H	C_6H_4(4-CH_3)	-	O	OEt	1	72
3	**6c**	H	C_6H_4(4-Br)	-	O	OEt	1	43
4	**6e**	H	C_6H_4(4-CH_2CH_3)	-	O	OEt	1	52
5	**7aa**	-	-	H	O	OEt	1	90
6	**7ab**	-	-	6-CH_3	O	OEt	1	67
7	**7ah**	-	-	H	O	OEt	2	40
8	**7ai**	-	-	H	O	OEt	3	37
9	**7be**	-	-	H	O	OH	2	82
10	**7bf**	-	-	H	O	OH	3	90

Table 2. Partial primary screening results of the synthesized compounds at a concentration of 500 nM



Entry	Compound	C	X	Y	n	% cell viability (at 500 nM)
1	**7ak**	H	S	OEt	0	92
2	**7al**	4-CH_3	S	OEt	0	78
3	**7ao**	6-OCH_3	S	OEt	0	71
4	**7au**	4-CH_3	S	OEt	1	62
5	**7ax**	6-OCH_2CH_3	S	OEt	1	65
6	**7az**	6-Cl	S	OEt	1	67
7	**7bh**	5,6-diCH_3	S	OH	1	81
8	**7bj**	4-CH_3	S	OH	1	78
9	**7bk**	6-Cl	S	OH	1	67
10	**7bl**	6-OCH_3	O	OK	1	76
11	**7bs**	H	O	OK	1	72
12	**7bt**	H	O	OK	2	33
13	**7bu**	H	O	OK	3	44

Experimental Section

Synthesis of Ureidothiazole or Ureidobenzothiazole ester 6 and 7 (General Procedure). To a solution of amine **3** (or **4**) (12 mmol) in tetrahydrofuran (6 mL) was added isocyanate(or isothiocyanate) **5** (12 mmol), and the mixture was heated to reflux under 80 °C for 2-10 h. The reaction mixture was cooled to room temperature and the precipitates were filtered, washed with cold ethyl ether, and then dried in air to afford the ester **6** (or **7**) (6-89% yields).

Hydrolysis of Ureidothiazole or Ureidobenzothiazole ester 6 and 7 (General Procedure). To a solution of the ester **6** (or **7**) (3.5 mmol) dissolved in methanol (3.3 mL/g) was added KOH (10.5 mmol). The reaction mixture was stirred for 30 min and poured onto water (30 mL/g) and heated to reflux under 100 °C for 3 h. The mixture was cooled in ice-bath and acidified (pH = 2) by addition of conc. HCl. The precipitates were filtered, washed with cold ethyl ether, and then dried in an oven at 40 °C to afford the corresponding acid **6** (or **7**) (Y=OH)(solid, 24-91% yields).

Synthesis of Ureidothiazole or Ureidobenzothiazole Potassium Salt (General Procedure). To a solution of the acid **6** (or 7) (0.26 mmol) dissolved in water (2.0 mL) was added a solution of KOH (0.26 mmol) in water (0.55 mL). The reaction mixture was stirred for 2 h at room temperature and filtrated using Millipore Sterivex-GV (0.22 μm membrane filter) to remove impurities and dust particles. The solvent was removed by freeze drying and the resulting solid was dissolved in water (0.8 mL). To the solution was dropwise added acetone (40 mL) and stirred for 2 h at room temperature. The precipitates were filtered, dried for 24 h at room temperature to afford the corresponding potassium salt **6** (or **7**) (Y=OK)(solid, 24-93% yields).

Supplementary Materials. Additional experimental procedures and biological screening method, the yields, melting point, ^{1}H NMR data for all the compounds, ^{13}C NMR, Mass and HPLC data for the representative compounds.

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