Article

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ACS Comb. Sci., **Just Accepted Manuscript** • DOI: 10.1021/acscombsci.6b00127 • Publication Date (Web): 19 Oct 2016

Solid-Phase Parallel Synthesis of *N*−Substituted-2-aminothiazolo[4,5-*b*] pyrazine Derivatives via Tandem Reaction of Isothiocyanate Terminated Resin with *o*-Bromo-2-Aminopyrazines

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ABSTRACT

A novel solid-phase synthesis methodology of *N*-substituted-2-aminothiazolo[4,5-*b*]pyrazine derivatives was developed. The key step in this synthesis strategy is the tandem reaction of isothiocyanate terminated resin **2** with *o*-bromo-2-aminopyrazine, affording cyclized 2-aminothiazolo[4,5-*b*]pyrazine resin **4**. To increase the diversity of our library, Suzuki coupling reaction was performed at the position C6. Further functionalization of 2-aminothiazolo[4,5-*b*]pyrazine core skeleton with various electrophiles such as alkyl halides, acyl chlorides, and sulfonyl chlorides and cleavage from the resin with TFA in DCM generated *N*-alkyl-, *N*-acyl-, and *N*-sulfonyl-2-aminothiazolo[4,5-*b*]pyrazine derivatives. The physicochemical properties and the polar surface areas of synthesized compounds were evaluated.

KEYWORDS: solid-phase, thiazolo[4,5-*b*]pyrazine, BOMBA resin, tandem reaction

INTRODUCTION

The solid-phase organic synthesis (SPOS) is one of the powerful tools in the synthesis of small organic molecules and building of libraries of compounds for drug discovery. In SPOS, an excess of reagents can be used to drive reaction to completion. Also products can be easily isolated by simple filtration and removed from solid support.[1] Other benefits of solid-phase are the ease of automation and the ability of polymer to "fish out" low concentrations of product molecules from excess of starting material.[2] Heterocyclic compounds usually serve as a backbone for hundreds of marketed drugs.[3] In this respect, we have been interested in thiazolo[4,5-*b*]pyrazine core skeleton. Even though thiazolo[4,5-*b*]pyrazine has not been investigated enough in the medicinal chemistry area,[4] we have focused on it because of its structural similarity with thiazolopyrimidine (Figure 1).

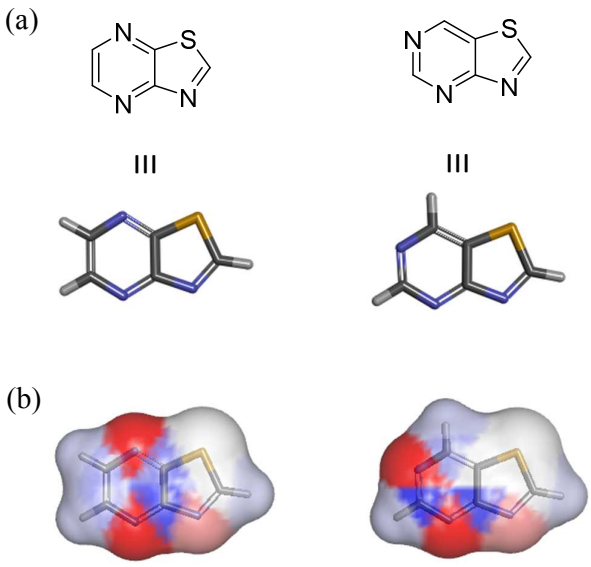


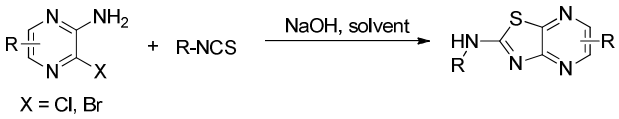
Figure 1. Comparison of energy minimized 3D structure (a) and polar surface area (b) of thiazolopyrimidine and thiazolopyrazine

Thiazolopyrimidine core skeleton has shown various biological activities in the medicinal chemistry area such as kinase inhibitors,[5] TRPV1 antagonists,[6] *E. coli* and *S. aureus* SecA inhibitors,[7] stearoyl-CoA desaturase (SCD) inhibitor,[8] adenosine A3 receptor antagonist.[9] Figure 1 shows similar structural features of thiazolopyrimidine and thiazolopyrazine (Figure 1a). The

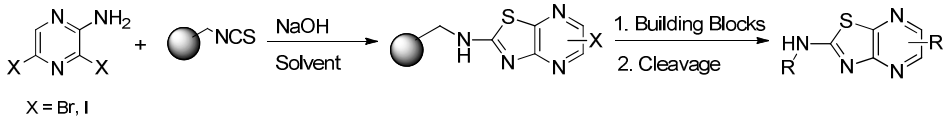
only difference is the position of nitrogen atom equipped in each core skeleton, and this positional difference causes different polar surface area (Figure 1b). In drug discovery, polar surface area is considered as a key factor to interact with target protein by non-covalent interaction, and positional difference of nitrogen atom has a different possibility for hydrogen bonding. In this respect, thiazolo[4,5-*b*]pyrazine was expected to exhibit various biological activities and good physicochemical properties.

Scheme 1. Synthetic Strategy for the Synthesis of *N*-Substituted-2-aminothiazolo[4,5-*b*]pyrazines

(a) Synthesis in Solution Phase



(b) Synthesis on Solid Phase

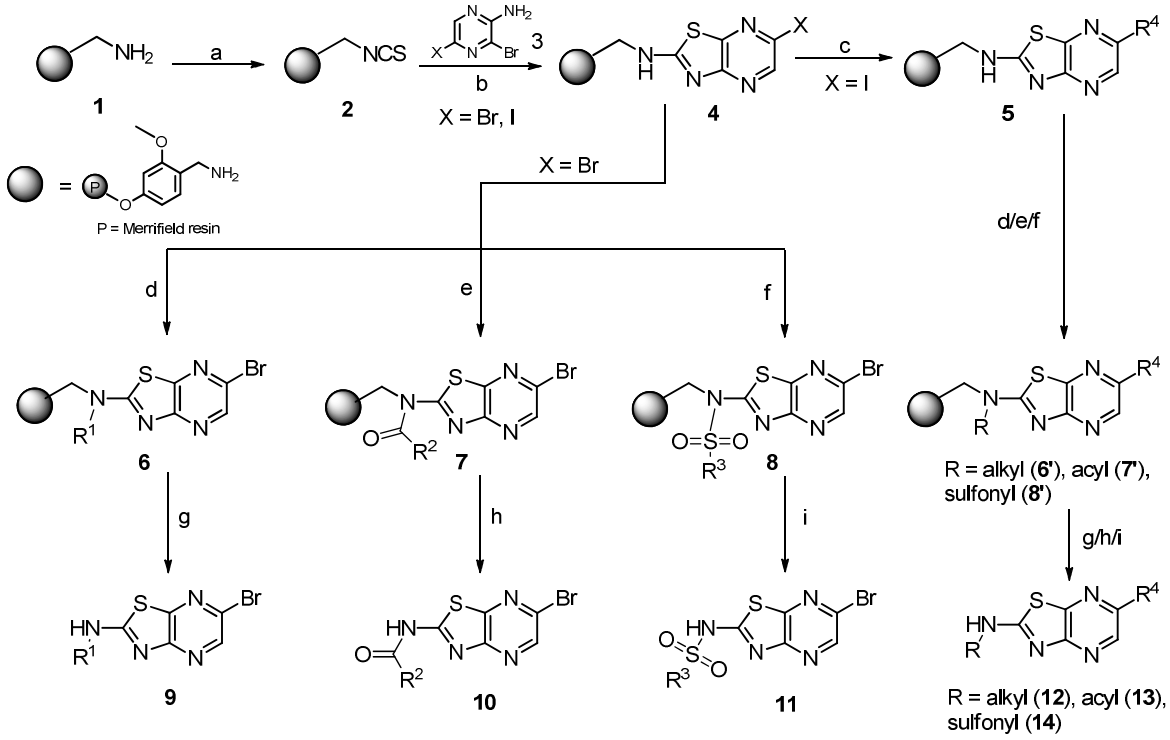


Several solution-phase synthesis routes of 2-aminothiazolopyrazines have been published.[10] We have been interested in the synthesis of 2-aminothiazolopyrazines as well. As a result, we reported solution-phase synthetic method of *N*-substituted-2-aminothiazolo[4,5-*b*]pyrazines via tandem reaction of *o*-aminohalopyrazines with isothiocyanates in 2012 (Scheme 1 (a)).[11] Even though various solution-phase synthesis methodologies have already been reported, no solid-phase synthesis approach has been reported until now. Accordingly, we aimed to develop an efficient synthesis route using solid-phase and construct a diverse library of compounds. Herein, we report an efficient synthetic methodology of 2-aminothiazolo[4,5-*b*]pyrazine on solid phase and its further functionalization to provide *N*-substituted-2-aminothiazolo[4,5-*b*]pyrazine derivatives based on the solution-phase synthetic route (Scheme 1 (b)).

RESULTS AND DISCUSSION

Chemistry

Scheme 2. Synthesis of *N*-Substituted-2-aminothiazolo[4,5-*b*]pyrazine Derivatives on Solid Phase[a]

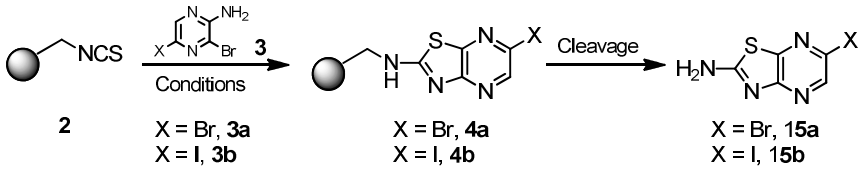


[a]Reaction conditions: (a) CS_2, *p*-TsCl, TEA, THF, room temperature, 18 h; (b) *o*-dihalo-2-aminopyrazine, NaOH, DMSO/THF or acetone, room temperature, 8 h; (c) $R^4B(OH)_2$, Cs_2CO_3, $Pd_2(dba)_3$, XPhos, 1,4-dioxane, H_2O, 110 °C, 24 h.; (d) alkyl halide, *t*-BuOK, THF, 60 °C, 16 h.; (e) acyl chloride, TEA, DMAP, THF, room temperature, 8 h.; (f) sulfonyl chloride, NaH, DMAP, THF, room temperature, 8 h; (g) TFA:DCM (1:4, v/v), 60 °C, 6 h; (h) TFA:DCM (1:9, v/v), room temperature, 6 h; (i) TFA:DCM (1:9, v/v), room temperature, 8 h.

The overall synthetic strategy for the synthesis of *N*-substituted-2-aminothiazolo[4,5-*b*]pyrazine derivatives is shown in Scheme 2. The synthesis route starts from the preparation of isothiocyanate terminated resin **2** from the 4-benzyloxy-2-methoxybenzylamine[12] (BOMBA) resin **1** by the treatment with Et_3N, CS_2, and *p*-TsCl.[13] The formation of the desired resin **2** was confirmed by attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR), showing typical isothiocyanate band at 2062 cm^{-1} (Figure S1 (b), Supporting Information). Next, the cyclization reaction of 2-aminothiazolo[4,5-*b*]pyrazine was performed. Before accomplishing

cyclization reaction on solid phase, model study in solution phase was performed (Table S1, Supporting Information). According to the results from the model study, resin **2** was reacted with 3,5-dibromo-2-aminopyrazine **3a** in the presence of NaOH in DMSO at room temperature (Table 1, entry 1).

Table 1. Results of the Optimization of Cyclization Reaction Conditions on Solid Phase[a]

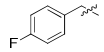
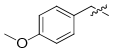
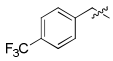
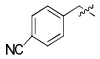
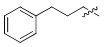
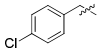


entry	X	base	solvent	temp. (°C)	time (h)	yield (%)[b]
1	Br	NaOH	DMSO	RT	8	trace
2	Br	NaOH	DMSO, THF	RT	8	56
3	I	NaOH	acetone	RT	12	60

[a]Reaction conditions: 3,5-dihalo-2-aminopyrazine (3.0 equiv) was treated with NaOH (5.0 equiv) in DMSO; after stirring for 20 min, reaction mixture was poured into a suspension of isothiacyanate terminated resin (1.0 equiv) in THF and mixture was shaken for 8 h.
[b]Three-step overall yield from BOMBA resin **1** (loading capacity of resin **1** is 0.91 mmol/g).

Unfortunately, desired cyclized product **15a** was obtained in trace amount because of low swelling property of DMSO into the lipophilic resin **2**. To solve this problem, we changed the solvent to a mixture of DMSO and THF in equal ratio to increase solvent system's swelling properties. As a result, **15a** was obtained in 56% three-step overall yield (Table 1, entry 2). Next, for better reactivity for further modification using Suzuki coupling, we replaced bromine to iodine at C6 position. From the result of the model study (Table S1, entry 7, Supporting Information), resin **2** was reacted with 3-bromo-5-iodopyrazin-2-amine **3b** in the presence of NaOH in acetone (Table 1, entry 3). Consequently, **15b** was obtained in 60% three-step overall yield. The cyclization reaction on solid phase was monitored by ATR-FTIR, and the absence of the isothiocyanate band and the presence of the imine stretching band at 1581 cm^{-1} confirmed the completion of the reaction (Figure S1 (c), Supporting Information).

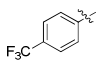
Table 2. Yields and Purities of the *N*-alkyl-2-aminothiazolo[4,5-*b*]pyrazine Derivatives

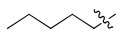
No	R^1	Yield (%)[a]	Purity (%)[b]	No	R^1	Yield (%)[a]	Purity (%)[b]
9a		39	100	**9i**		31	100
9b		29	100	**9j**		39	100
9c		35	100	**9k**		35	100
9d		33	100	**9l**		25	100
9e		47	100	**9m**		38	100
9f		27	100	**9n**		50	100
9g		33	100	**9o**		43	100
9h		34	100	**9p**		20	100

[a]Four-step overall yield from BOMBA resin **1** (loading capacity of resin **1** is 0.91 mmol/g).
[b]All the purified products were checked by LC/MS.

For the diversification of the obtained 2-aminothiazolo[4,5-*b*]pyrazine core skeleton, various electrophiles such as alkyl halides, acyl chlorides, and sulfonyl chlorides were introduced. First, we introduced alkyl halides in the presence of *t*-BuOK in THF at 60 °C for 16 h. The cleavage from the supporting resin **6** with TFA in DCM (1:4, v/v) at 60 °C for 6 h afforded the desired product. The crude product mixture was purified by flash chromatography (hexane/ethyl acetate) and trituration with diethyl ether and ethyl acetate (9:1, v/v) to afford *N*-alkyl-2-aminothiazolo[4,5-*b*]pyrazine derivatives **9** in good overall yields and high purities, as listed in Table 2.

Table 3. Yields and Purities of the *N*-acyl-2-aminothiazolo[4,5-*b*]pyrazine Derivatives

No	R^2	Yield (%)[a]	Purity (%)[b]	No	R^2	Yield (%)[a]	Purity (%)[b]
10a		43	100	**10l**		41	100
10b		52	100	**10m**		47	100

10c		47	100	**10n**		47	100
10d		46	100	**10o**		49	100
10e		35	100	**10p**		29	100
10f		50	100	**10q**		19	100
10g		51	100	**10r**		33	100
10h		49	100	**10s**		51	100
10i		46	100	**10t**		39	100
10j		48	100	**10u**		37	100
10k		55	100	**10v**		26	100

[a]Four-step overall yield from BOMBA resin **1** (loading capacity of resin **1** is 0.91 mmol/g).
[b]All the purified products were checked by LC/MS.

Table 4. Yields and Purities of the *N*-sulfonyl-2-aminothiazolo[4,5-*b*]pyrazine Derivatives

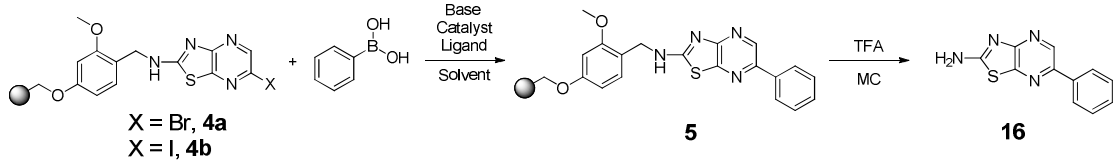
No	R^3	Yield (%)[a]	Purity (%)[b]	No	R^3	Yield (%)[a]	Purity (%)[b]
11a		19	100	**11j**		18	100
11b		14	100	**11k**		17	100
11c		14	100	**11l**		13	100
11d		24	100	**11m**		17	100
11e		20	100	**11n**		14	100
11f		29	100	**11o**		19	100
11g		21	100				
11h		21	100	**11p**		14	100
11i		24	100	**11q**		9	100

[a]Four-step overall yield from BOMBA resin **1** (loading capacity of resin **1** is 0.91 mmol/g).
[b]All the purified products were checked by LC/MS.

Next, 2-aminothiazolo[4,5-*b*]pyrazine resin **4a** was diversified with various acyl chlorides by acylation reaction in the presence of Et_3N, 4-dimethylaminopyridine (DMAP), and THF for 8 h at room temperature. The reaction was also monitored by ATR-FTIR, showing increasing intensity of the amide band at 1670 cm^{-1} (Figure S1 (e), Supporting Information). The cleavage of the acylated compound from resin **7** by the treatment with TFA in DCM (1:9, v/v) for 6 h afforded desired *N*-acyl-2-aminothiazolo[4,5-*b*]pyrazine derivatives **10** in high yields and purities. The LC/MS spectra of the crude product mixture (Figure S2, Supporting Information) showed fair purity without further purification after the cleavage. Thus, purification of acylated compounds was proceeded by trituration with diethyl ether and ethyl acetate (4:1, v/v) affording acylated compounds in good yields and high purities (Table 3).

Further diversification was proceeded by the reaction of sulfonyl chlorides with 2-aminothiazolo[4,5-*b*]pyrazine resin **4a** at room temperature for 8 h in THF in the presence of NaH as the base and DMAP. Reaction monitoring by ATR-FTIR showed the increase in the intensity of the sulfonamide band at 1364 and 1166 cm^{-1} (Figure S1 (f), Supporting Information). By the cleavage of the *N*-sulfonyl-2-aminothiazolo[4,5-*b*]pyrazine resin **8** with TFA in DCM (1:9, v/v) for 8 h at room temperature, *N*-sulfonyl-2-aminothiazolo[4,5-*b*]pyrazine derivatives **11** were obtained in good yields and high purities as listed in Table 4.

Table 5. Results of the Optimization of Suzuki Coupling Reaction Conditions on Solid Phase[a]



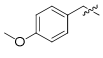
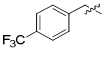
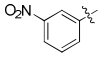
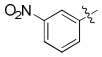
entry	X	base	catalyst	ligand	solvent	temp. (°C)	time (h)	yield (%) [b]
1	Br	Cs_3CO_3	$Pd_2(dba)_3$ 10 mol%	XPhos 20 mol%	1,4-dioxane : H_2O 9 : 1	110	16	NR
2	I	Cs_3CO_3	$Pd_2(dba)_3$ 10 mol%	XPhos 20 mol%	1,4-dioxane : H_2O 9 : 1	110	20	20

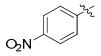
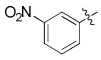
3	I	Cs_3CO_3	$Pd_2(dba)_3$ 20 mol%	XPhos 40 mol%	1,4-dioxane : H_2O 9 : 1	110	24	42

[a]Reaction conditions: To a suspension of 2-aminothiazolo[4,5-*b*]pyrazine resin **4** (1.0 equiv) in 1,4-dioxane, were added successively $Pd_2(dba)_3$ (20 mol%), XPhos (40 mol%), phenylboronic acid (3.0 equiv), and Cs_2CO_3 (5.0 equiv) dissolved in H_2O. Reaction mixture was shaken at 110 °C for 24 h. NR – No Reaction.
[b]Four-step overall yield from BOMBA resin **1** (loading capacity of resin **1** is 0.91 mmol/g).

For additional diversification, Suzuki coupling was performed. First, reaction conditions were optimized in solution phase giving high yield of the coupled product by the reaction of 6-bromo-thiazolo[4,5-*b*]pyrazin-2-amine with phenyl boronic acid, tris(dibenzylideneacetone)dipalladium (0) ($Pb_2(dba)_3$) as the catalyst, XPhos as the ligand in the presence of cesium carbonate (Cs_2CO_3) in 1,4-dioxane:H_2O (9:1, v/v) for 20 h at 110 °C (Table S2, Supporting Information). However, these optimized conditions were not successful on solid phase as it shown in Table 5, entry 1. Thus, we decided to change bromine atom to more reactive iodine atom at C6 position and run reaction under the same conditions. Fortunately, changing halogen to iodine increased the reactivity and Suzuki coupling was carried out on solid phase under the same reaction conditions and the four-step overall yield was 20 % (Table 5, entry 2).

Table 6. Yields and Purities of 6-aryl-*N*-substituted-2-aminothiazolo[4,5-*b*]pyrazine Derivatives

No	R^1	R^4	Yield (%)[a]	Purity (%)[b]	No	R^2	R^4	Yield (%)[a]	Purity (%)[b]
12a			22	100	**13a**			39	100
12b			32	100	**13b**			31	100
12c			29	100	**13c**			15	100
12d			7	96	**13d**			40	100
12e			13	100	**13e**			32	100
12f			16	100	**13f**			30	100
12g			9.5	100	**13g**			37	100
12h			NR	-	**13h**			NR	-

No	R^3	R^4	Yield (%)[a]	Purity (%)[b]	No	R^3	R^4	Yield (%)[a]	Purity (%)[b]
14a			5	100	**14d**			29	85
14b			9	100	**14e**			NR	-
14c			10	100					

[a]Five-step overall yield from BOMBA resin **1** (loading capacity of resin **1** is 0.91 mmol/g).
[b]All the purified products were checked by LC/MS. NR – No Reaction.

However, the conversion of starting material was not completed. Thus, we increased the ratio of catalyst and reaction time. With new conditions, we obtained Suzuki coupled product with 42 % four-step overall yield (Table 5, entry 3). To increase the diversity of compound, Suzuki coupling was accomplished with different boronic acids. In the case of aryl boronic acid (phenyl boronic acids and 3-thiophenyl boronic acid) the Suzuki coupling proceeded with good yields and high purities. However, with aliphatic ethyl boronic acid there was no reaction (Table 6, **12h**, **13h**, and **14e**). Next, the functionalization of 6-aryl-2-aminothiazolo[4,5-*b*]pyrazine resin **5** with various alkyl halides, acyl chlorides, and sulfonyl chlorides was carried out as it was explained before (d/e/f, Scheme 2) to afford corresponding *N*-substituted resins **6′**, **7′**, and **8′** respectively. The subsequent treatment of each resin with TFA in DCM afforded desired *N*-alkyl-6-arylthiazolo[4,5-*b*]pyrazin-2-amine **12**, 6-aryl-*N*-acyl-2-aminothiazolo[4,5-*b*]pyrazine **13**, and 6-aryl-*N*-sulfonyl-2-aminothiazolo[4,5-*b*]pyrazine **14** derivatives (Table 6).

Displayed 3D structure and calculation for physicochemical properties

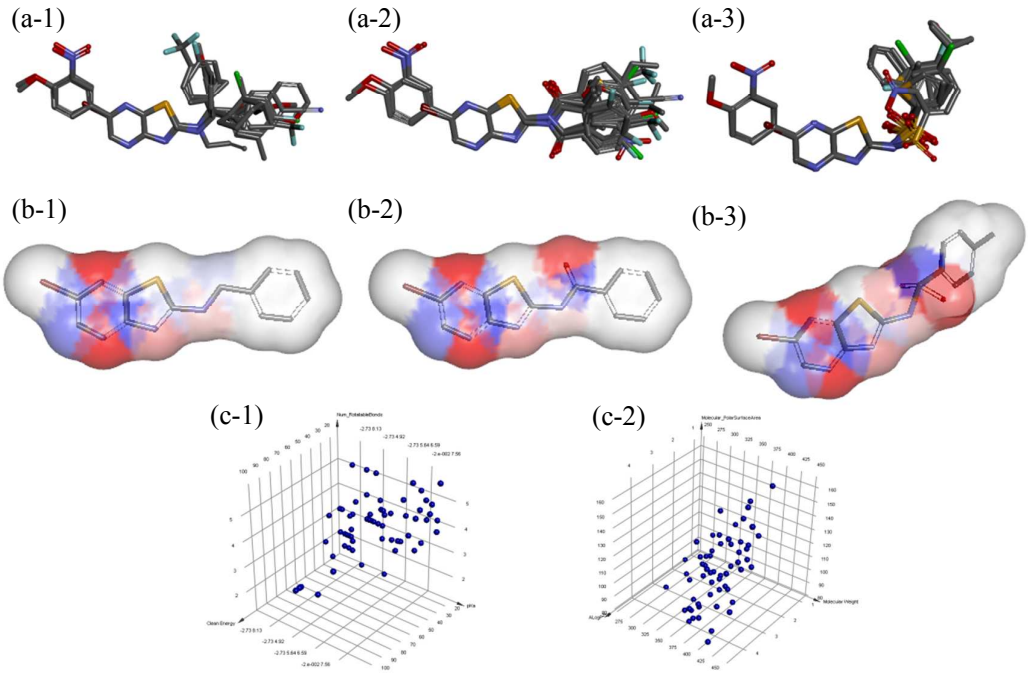


Figure 2. Alignment of energy minimized 3D structure of alkylated compounds **9** and **12** (a-1), acylated compounds **10** and **13** (a-2), and sulfonylated compounds **11** and **14** (a-3); polar surface areas of **9a** (b-1), **10a** (b-2), **11a** (b-3); physicochemical properties in 3D plots: number of rotatable bonds, clean energy, and pKa (c-1); polar surface area, calculated LogP, and molecular weight (c-2)

Lastly, 3D structural difference, polar surface areas, and physicochemical properties are displayed in Figure 2. As it shown in Figure 2a, each energy minimized 3D structure is aligned with pyrazine ring of thiazolo[4,5-*b*]pyrazine and the structure diverged widely by various building blocks in 3D space. Next, the polar surface areas of representative alkylated product **9a**, acylated product **10a**, and sulfonylated product **11a** are shown in Figure 2b. It is clear that the polar surface area of each functionalized thiazolo[4,5-*b*]pyrazine is different, indicating that this thiazolo[4,5-*b*]pyrazine derivatives can interact with different biomarkers. In addition, the physicochemical properties (number of rotatable bonds, clean energy, pKa, polar surface area, calculated LogP, and molecular weight) of the constructed thiazolo[4,5-*b*]pyrazine library are displayed in 3D chemical plots showing that the physicochemical properties are arranged widely. (Figure 2c).

Molecular docking study

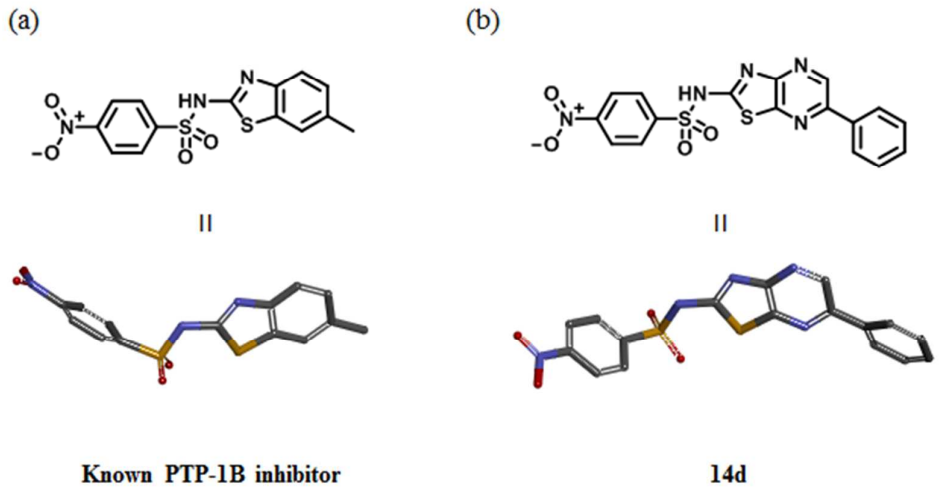


Figure 3. Comparison of already known PTP-1B inhibitor and **14d**

For a biological docking evaluation, we conducted virtual screening with protein phosphatase 1B (PTP-1B, PDB Code : 2F71) by discovery studio 2016 (DS 2016). PTP-1B is associated with type 2 diabetes mellitus (T2DM) which is metabolic disease characterized by insulin resistance in peripheral tissues (liver, muscle, and adipose) and damaged insulin secretion by the pancreas.[14] Navarrete-Vazquez reported that 2-arylsulfonylaminobenzothiazoles showed anti-hyperglycemic activity by inhibition of PTP-1B.[15] As can be seen in Figure 3, most potent inhibitor (Figure 3a) to PTP-1B reported by Navarrete-Vazquez has a similar structural features with our 2-sulfonamido-thiazolo[4,5-*b*]pyrazine **14d**, promising that 2-sulfonamido-thiazolo[4,5-*b*]pyrazine **14d** might play as potent inhibitor to PTP-1B. For this reason, we investigated molecular docking study with **14d** to PTP-1B.

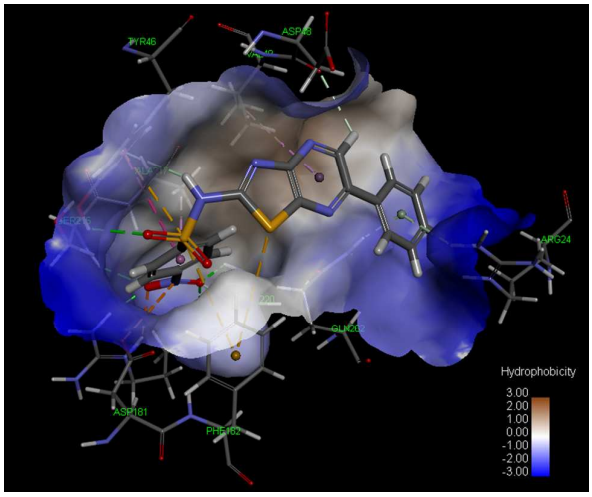


Figure 4. A superimposed view of **14d** binding to the active site of PTP-1B

According to the molecular docking result, **14d** interacted with the active site of PTP-1B in similar way as already known PTP-1B inhibitor (Figure 4). It seems clear that nitro group in **14d** plays important role in binding PTP-1B with GLY220, and ARG221 through hydrogen bonding in hydrophilic binding pocket. The oxygen in sulfonamide group also makes hydrogen bonding with TYR46. It is interesting to note that the hydrogen at C5 position makes carbon hydrogen bonding with ASP48 since this hydrogen might be quite acidic because of adjacent nitrogen atom in pyrazine ring. Furthermore, the phenyl group introduced by Suzuki coupling makes pi-donor hydrogen bonding with ARG24.

Conclusion

In conclusion, we established an effective solid-phase synthetic methodology to synthesize *N*-substituted-2-aminothiazolo[4,5-*b*]pyrazines. Diversification of the obtained core skeleton was accomplished by functionalization with alkyl halides, acid chlorides, and sulfonyl chlorides, as well as by introducing Suzuki coupling at the C6 position. After the functionalization of 2-aminothiazolo[4,5-*b*]pyrazines, the supporting resin was cleaved with TFA in DCM, affording the desired final compounds in good yields and purities. To investigate the potential of the synthesized compounds as a drug, structural diversity, polar surface area, and physicochemical properties were evaluated. As a result, we confirmed that our library of compounds has high structural diversity in 3D space resulted by functionalization, and difference polar surface area, indicating different possibilities to interact with biomarkers. The physicochemical properties of the library are widely arranged in 3D chemical plots. Finally, molecular docking study clearly showed the possibility of the **14d** to be used as a PTP-1B inhibitor of type 2 diabetes mellitus (T2DM). We will explore biological evaluation with *N*-substituted-2-aminothiazolo[4,5-*b*]pyrazines in hand to extract potent PTP-1B inhibitor in our future work.

EXPERIMENTAL PROCEDURES

General Procedure for Synthesis All the chemicals were of reagent grade and used as purchased. Reactions were monitored by TLC analysis using Merck silica gel 60 F-254 thin layer plates (for solution-phase synthesis) and by ATR-FTIR spectrometer (for solid-phase synthesis). The crude product mixtures were purified by flash chromatography system using Isolera One (Biotage). Flash column chromatography was carried out on Merck silica gel 60 (230−400 mesh). ^{1}H NMR and ^{13}C NMR spectra were recorded in δ units relative to deuterated solvent as an internal reference using a 500 MHz NMR spectrometer (Bruker). For liquid chromatography tandem mass spectrometry (ESI, PDA detection), 6460 Triple Quad LC/MS (Agilent) was used. High-resolution mass spectrometry was performed using a 6550 iFunnel Q-TOF LC/MS system (Agilent).

Representative Procedure for the Preparation of the Isothiocyanate Terminated Resin 2 To a mixture of BOMBA resin **1** (5 g, 4.55 mmol) in THF (25 mL), Et_3N (4.6 g, 46 mmol) and CS_2 (2 g, 27.3 mmol) were added at 0 °C, resulting in a suspension. After shaking the suspension for 3 h at room temperature, *p*-TsCl (4.34 g, 22.7 mmol) was added slowly at 0 °C, and the suspension was shaken for 12 h at room temperature. Isothiocyanate terminated resin **2** was filtered and washed successively with THF, MeOH, H_2O, MeOH (×2), and DCM (×2), and dried under high vacuum. Single-Bead ATR-FTIR: 3028, 2923, 2062 (N=C=S), 1608, 1507, 1420, 1268, 1195, 1088, 820, 757 cm^{-1}.

Representative Procedure for the Preparation of the Cyclized 2-aminothiazolo[4,5-b]pyrazine Resin 4a 3,5-dibromo-2-aminopyrazine (3.43 g, 13.6 mmol) was dissolved in DMSO (10 mL) followed by adding NaOH (0.9 g, 22.6 mmol). The mixture was stirred for 20 min at room temperature. Then, the reaction mixture was poured into a suspension of isothiocyanated resin **2** (5 g, 4.55 mmol) in THF (10 mL). The reaction mixture was shaken for 8 h at room temperature, filtered, and washed successively with DCM, MeOH, H_2O, MeOH (×2) and DCM (×2), and

dried under high vacuum. Single-Bead ATR-FTIR: 3021, 2920, 1492, 1581 (N=C), 1354, 1282, 1186, 1159, 815, 758 cm^{-1}.

In the case of resin **4b**, acetone was used instead of DMSO and THF.

Representative Procedure for the Suzuki Coupling reaction

Preparation of 6-phenylthiazolo[4,5-b]pyrazin-2-amine resin 5a Reaction was carried out under an inert atmosphere. To a suspension of 2-aminothiazolo[4,5-*b*]pyrazine resin **4b** (1.2 g, 1.09 mmol) in 1,4-dioxane (5.4 mL), $Pd_2(dba)_3$ (0.2 g, 0.22 mmol), XPhos (0.21 g, 0.44 mmol), phenylboronic acid (0.4 g, 3.2 mmol), and Cs_2CO_3 (1.78 g, 5.4 mmol) dissolved in H_2O (0.6 mL) were added. The reaction mixture was shaken for 24 h at 110 °C and cooled to room temperature. The resin was filtered and washed successively with DCM, MeOH, H_2O, MeOH (×2), and DCM (×2), and dried under high vacuum.

Representative Procedure for the Alkylation Reaction

Preparation of N-benzyl-2-aminothiazolo[4,5-b]pyrazine Resin 6a To a mixture of cyclized resin **4a** (0.220 g, 0.2 mmol) in THF (3 ml), *t*-BuOK (0.112 g, 1.0 mmol) was added, and the reaction mixture was shaken for 1 h at room temperature. After 1 h benzyl chloride (0.12 mL, 1.0 mmol) was added, and the temperature was increased to 60 °C. The reaction mixture was shaken for 16 h. The resin was filtered and washed successively with DCM, MeOH, H_2O, MeOH (×2), and DCM (×2), and dried under high vacuum. Single-Bead ATR-FTIR: 3026, 2920, 1603, 1574, 1492, 1348, 1193, 819, 758, 737 cm^{-1}.

Representative Procedure for the Acylation Reaction

Preparation of N-benzoyl-2-aminothiazolo[4,5-b]pyrazine Resin 7a To a suspension of cyclized resin **4a** (0.220 g, 0.2 mmol) in THF (3 mL), Et_3N (0.14 mL, 1.0 mmol), benzoyl chloride (0.12 mL, 1.0 mmol), and DMAP (cat. amount) were added successively. The reaction mixture was shaken for 8 h at room temperature. *N*-benzoyl-2-aminothiazolopyrazine resin **7a** was filtered and washed successively with DCM, MeOH, H_2O, MeOH (×2), and DCM (×2), and dried under

high vacuum. Single-Bead ATR-FTIR: 3021, 2923, 1670 (amide), 1654, 1600, 1507, 1449, 1375, 1192, 961, 815, 759 cm^{-1}.

Representative Procedure for the Sulfonylation Reaction

Preparation of N-(6-bromothiazolo[4,5-b]pyrazin-2-yl)-4-methylbenzenesulfonamide Resin 8a To a suspension of cyclized resin **4a** (0.220 g, 0.2 mmol) in THF (3 mL), NaH (0.04 g, 1.0 mmol, 60% suspension in oil), *p*-TsCl (0.190 g, 1.0 mmol), DMAP (cat. amount) were added successively. The reaction mixture was shaken for 8 h at room temperature. Sulfonylated resin **8a** was filtered and washed successively with DCM, MeOH, H_2O, MeOH (×2), and DCM (×2), and dried under high vacuum. Single-Bead ATR-FTIR: 3026, 2925, 1603, 1507, 1446, 1364 (S=O), 1186, 1166, 1109, 814, 757 cm^{-1}.

Representative Procedure for the Preparation of N-benzyl-6-bromothiazolo[4,5-b]pyrazin-2-amine 9a Resin **6a** after alkylation (0.245 g, 0.22 mmol) was treated with a mixture of TFA and DCM (1:4, v/v, 2 mL) and shaken for 6 h at 60 °C. The resin was filtered and washed with DCM and MeOH (×3), and organic filtrate was collected and evaporated. The residue was dissolved in ethyl acetate, washed with H_2O, and neutralized to pH 7 with saturated aqueous $NaHCO_3$ solution. The organic layer was dried with anhydrous $MgSO_4$ and evaporated. The crude mixture was purified by column chromatography on silica gel and triturated with diethyl ether and ethyl acetate (9:1, v/v) affording *N*-benzyl-6-bromothiazolo[4,5-*b*]pyrazin-2-amine **9a** (39%, four-step overall yield). ^{1}H NMR (500 MHz, CDCl3) δ 8.37 (s, 1H), 7.42–7.33 (m, 5H), 7.26 (s, 1H), 4.73 (s, 2H). ^{13}C NMR (126 MHz, CDCl3) δ 168.67, 157.65, 149.46, 142.91, 136.07, 130.13, 129.02, 128.35, 127.97, 48.84. LC–MS (ESI): m/z = 321.0 $[M+H]^+$. HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{12}H_9BrN_4S$ 320.9804; found 320.9813.

Representative Procedure for the Preparation of N-(6-bromothiazolo[4,5-b]pyrazin-2-yl)benzamide 10a Acylated resin **7a** (0.220 g, 0.2 mmol) was treated with a mixture of TFA and DCM (1:9, v/v, 2 mL) and shaken for 6 h at room temperature. The resin was filtered and

washed with DCM and MeOH (×3), and organic filtrate was collected and evaporated. The crude mixture was purified by trituration with diethyl ether and ethyl acetate (4:1, v/v) solvent mixture and dried under high vacuum. The resulting product was brown color solid (43%, four-step overall yield). ^{1}H NMR (500 MHz, DMSO) δ 13.53 (s, 1H), 8.82 (s, 1H), 8.17 (d, *J* = 8.0 Hz, 2H), 7.72 (t, *J* = 7.8 Hz, 1H), 7.61 (t, *J* = 7.7 Hz, 2H). ^{13}C NMR (126 MHz, $CDCl_3$ + $C_2HF_3O_2$) δ 169.82, 167.76, 150.15, 149.18, 144.36, 136.96, 136.66, 130.71, 130.09, 129.63. LC-MS (ESI): m/z = 335.0 $[M+H]^+$. HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{12}H_7BrN_4OS$ 334.9597; found 334.9597.

Representative Procedure for the Preparation of N-(6-bromothiazolo[4,5-b]pyrazin-2-yl)-4-methylbenzenesulfonamide 11a Sulfonylated resin **8a** (0.235 g, 0.21 mmol) was treated with a mixture of TFA and DCM (1:9, v/v, 2 mL) and shaken for 8 h at room temperature. The resin was filtered and washed with DCM and MeOH (×3), and organic filtrate was collected and evaporated. The residue was dissolved in ethyl acetate, washed with H_2O, and neutralized to pH 7 with saturated aqueous $NaHCO_3$ solution. The organic layer was dried with anhydrous $MgSO_4$ and evaporated. The crude mixture was purified by column chromatography on silica gel and triturated with diethyl ether and ethyl acetate (9:1, v/v) affording desired product *N*-(6-bromothiazolo[4,5-*b*]pyrazin-2-yl)-4-methylbenzenesulfonamide **11a** (19%, four-step overall yield). ^{1}H NMR (500 MHz, DMSO) δ 8.22 (s, 1H), 7.69 (d, *J* = 8.2 Hz, 2H), 7.26 (d, *J* = 8.2 Hz, 2H), 2.32 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$ + $C_2HF_3O_2$) δ 166.03, 146.96, 145.83, 143.74, 142.31, 135.21, 135.16, 130.78, 127.42, 21.82. LC-MS (ESI): m/z = 384.9 $[M+H]^+$. HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{12}H_9BrN_4O_2S_2$ 384.9423; found 384.9434.

In the case of ^{1}H NMR of *N*-sulfonyl-2-aminothiazolo[4,5-*b*]pyrazine derivatives, ^{1}H NMR was checked in different solvents (DMSO-D6, $CDCl_3$, THF-D8, acetone-D6), but still no NH proton was observed.

Molecular docking

The molecular docking study was performed by mean of CDOCKER and Flexible Docking using the DS CHARMm function and 3D structure of PTP-1B (PDB Code : 2F71).

Acknowledgment This study was supported by the Bio & Medical Technology Development Program of the National Research Foundation funded by the Ministry of Science, ICT & Future Planning (No. 2014M3A9A9073847), the Ministry of Trade, Industry & Energy, and the Korean Institute for Advancement of Technology through the industrial infrastructure program for fundamental technologies (Grant Number M0000338).

Supporting Information Available: Full analytical data of compounds, along with the copies of ^{1}H NMR, ^{13}C NMR, LC/MS, and HRMS spectra of all the synthesized compounds and ATR-FTIR spectrum of the corresponding resins; this material is available free of charge via the Internet at http://pubs.acs.org.

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Graphical Abstract

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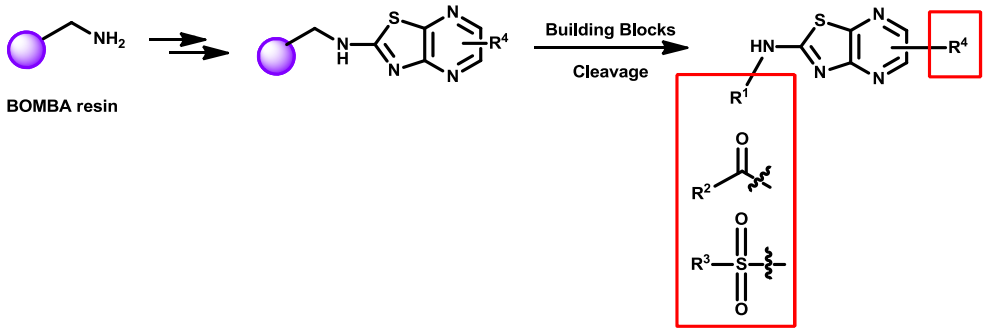

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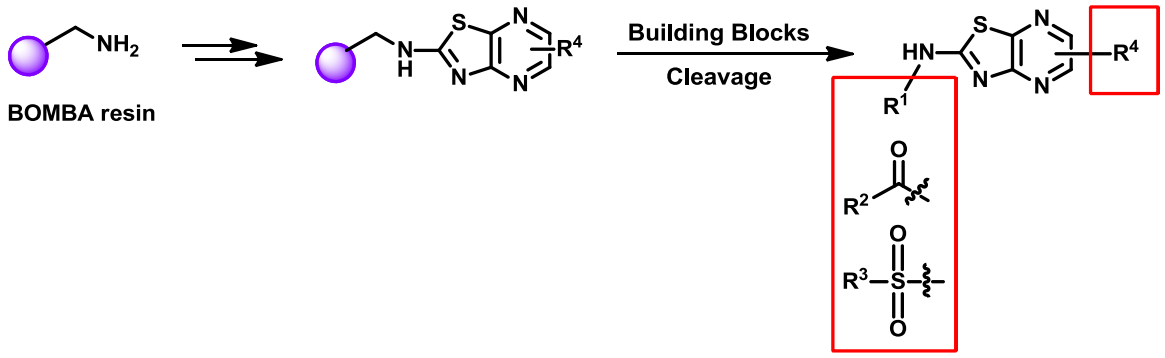

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