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Identification of a novel hormone sensitive lipase inhibitor with a reduced potential of reactive metabolites formation

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ABSTRACT

Hormone sensitive lipase (HSL) has emerged as an attractive target for the treatment of dyslipidemia. We previously reported compound **1** as a potent and orally active HSL inhibitor. Although an attractive profile was demonstrated, subsequent studies revealed that compound **1** has a bioactivation liability. The oxygen-carbon linker in compound **1** was identified as being potentially responsible for reactive metabolite formation. By exchanging of this susceptible fragment was feasible, and a benzanilide derivative **6b** with a decreased bioactivation liability was obtained. Further modification of the novel benzanilide scaffold resulted in the identification of compound **24b**. Compound **24b** exhibited potent HSL inhibitory activity (IC_{50} = 2 nM) with a significantly reduced bioactivation potential. Oral administration of compound **24b** exhibited an antilipolytic effect on rats at 3 mg/kg.

1. Introduction

Hormone sensitive lipase (HSL) is an intracellular neutral lipase that catalyzes hydrolysis of broad substrates such as tri-, di-, and monoacylglycerol (TG, DG and MG), cholesterylester, retinyl ester and numerous water soluble ester substrates.[1] HSL contains an α/β-hydrolase fold[2] and a catalytic triad of serine, aspartic acid, and histidine,[3] which are essential for catalysis.[4] The highest expression of HSL is observed in adipose tissues (ATs) where it catalyzes lipolysis of stored triglyceride (TG); one TG molecule is sequentially broken down into one glycerol and three free fatty acids (FFAs). The cellular concentration of FFAs is tightly controlled by the balance between lipolysis of TG and esterification of FFAs by several hormones depending on energy demand.[5] In the fasted state, HSL is activated in response to catecholamines and FFAs are subsequently released into circulation as an energy source for most tissues.[6]

FFAs have a pathophysiological role in dyslipidemia as well as a physiological role in energy production. Elevation of plasma FFA level is associated with obesity and insulin resistance that induce enlargement of AT mass and attenuation of insulin-mediated AT lipolysis metabolism, which could cause dysregulation of lipolysis. Furthermore, the increased FFA flux could impair lipid profiles by enhancing hepatic production of very-low-density lipoprotein (VLDL).[7] HSL inhibition could be a promising therapeutic approach for dyslipidemia, however, the reports of HSL inhibitors have been limited.[8–17]

We previously reported that compound **1** exerted HSL inhibitory activity with an IC_{50} value of 7 nM and an in vivo antilipolytic effect at 3 mg/kg in rats.[18] Despite potent in vitro and in vivo activities, subsequent studies revealed that compound **1** has a potential to form reactive metabolites. Reactive metabolite formation could cause organ toxicity and carcinogenesis, since these metabolites covalently bind to biological macromolecules such as protein and DNA.[19] Therefore, minimizing the potential to form reactive metabolites could be required.[20]

The propensity of forming reactive metabolites was generally assessed by incubation of compounds with glutathione (GSH) in the presence of liver microsomes.[21] In the reactive metabolite trapping assay, a GSH-derived adduct of compound **1** was detected (Fig. 1).[22] Its structural elucidation using LC-MS/MS analysis provided that the site of GSH conjugation was tentatively assigned to the benzyl carbon. On the basis of the results, the proposed

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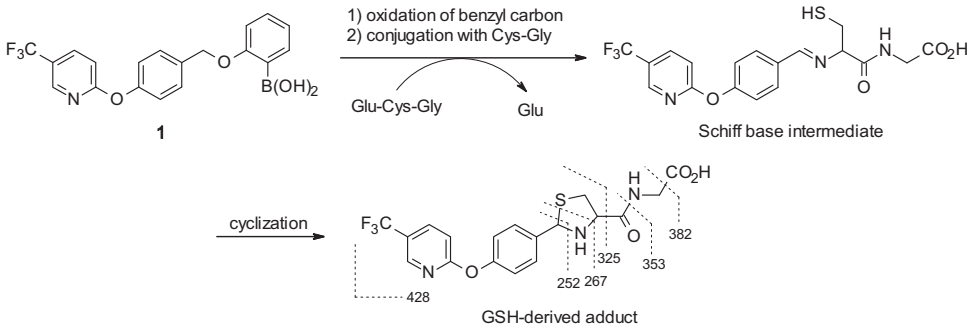


Fig. 1. Proposed mechanism of the GSH-derived adduct formation of **1**.

mechanism of the GSH-derived adduct formation involves initial oxidation of the benzyl carbon by cytochrome P450, followed by conjugation with a primary amino group of a cystein moiety that is released from GSH. The resulting Schiff base intermediate ring-contracts to a thiazolidine leading to the GSH-derived adduct.[23,24] We focused on exchanging from the susceptible fragment, namely, the oxygen-carbon linker, to an alternative fragment to circumvent the reactive metabolite liability while maintaining HSL inhibitory activity.

Herein, we report the identification of novel benzanilide derivatives that showed potent in vitro and in vivo activities with a decreased potential for forming reactive metabolites.

2. Chemistry

Scheme 1 depicts the synthesis of derivatives **3**, **6a–c** and **8**. Acylation of **2** furnished **3**. Condensation of corresponding acids (**4**[25] or **7**) with anilines (**5a** or **5b**) yielded compound **6a**, **6c** and **8**. Deprotection of pinacol group in **6a** led to boronic acid derivative **6b**.

Scheme 2 describes the synthesis of derivatives **11**, **13a–b**, **16a–b**, **19a–b** and **22a–c**. Condensation of 2-chloro-5-(trifluoromethyl) pyridine **9** with corresponding phenols followed by hydrolysis gave intermediates **10b**, **12a** and **12b**, which were converted to **11**, **13a** and **13b** in a two-step sequence. The intermediates **15a** and **15b** were synthesized from 2-cyano-5-fluoropyridine **14** by condensation and a following two-step hydrolysis. Compounds **16a** and **16b** were obtained by acylation of **5a** with the intermediates **15a** and **15b**. Compounds **19a** and **19b** were synthesized by hydrolysis of **17**[26] and following condensation. Compounds **22a–c** were prepared in a similar manner to that of compound **11**.

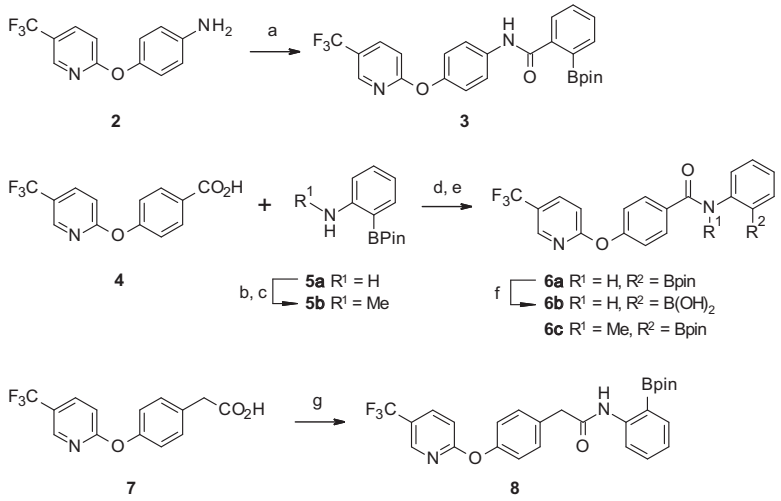
Scheme 3 illustrates the synthesis of compounds **24a–f**. Condensation of carboxylic acid **17** with corresponding anilines led to compounds **23a–f**, followed by a two-step deprotection of the pinacol group via trifluoroborate intermediates[27] afforded compounds **24a–f**.

3. Results and discussion

The inhibitory activity of the synthesized compounds against HSL was measured by a colorimetric assay using human HSL fractions and *p*-nitrophenyl butyrate (PNPB) as a substrate.[8]

In terms of efficient screening, we initially synthesized and evaluated derivatives as a boronate ester since the inhibitory activity of a boronic acid was considered to be comparable to that of a corresponding ester.[18] As seen in Table 1, replacement of the oxygen-carbon linker with an amide linker led to compound **6a** with an IC_{50} value of 0.18 μM. Conversion of the boronate ester **6a** to a boronic acid **6b** resulted in a comparable HSL inhibitory activity. *N*-substituted amide **6c** or the reverse amide **3** significantly deteriorated the inhibitory activity compared with **6a**. Expanding the amide linker also resulted in a loss of the inhibitory activity (**8**). As expected, GSH-derived adduct formation in the boronic acid **6b** was not detected by the reactive metabolite trapping assay with GSH. Therefore, boronic acid **6b** was identified as a lead compound, and this benzanilide scaffold was selected for further exploration.

We explored the left hand moiety of the novel derivatives as boronate ester derivatives (Table 2). First, the *m*-substituted analog



Scheme 1. Reagents and conditions: (a) 2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoic acid, EDC·HCl, HOBT, DMF; (b) 1*H*-benzotriazole-1-methanol, EtOH; (c) $NaBH_4$, THF; (d) oxalyl chloride, DMF (cat.), CH_2Cl_2; (e) **5a** or **5b**, DIPEA, CH_2Cl_2; (f) $NaIO_4$, THF, H_2O, then 1 M HCl; (g) **5a**, EDC·HCl, HOBT, NMM, CH_2Cl_2.