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Metabolic activation of *N*-thiazol-2-yl benzamide as glucokinase activators: Impacts of glutathione trapping on covalent binding

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ABSTRACT

Glucokinase activators (GKAs) are currently under investigation as potential antidiabetic agents by many pharmaceutical companies. Most of GKAs reported previously possess *N*-aminothiazol-2-yl amide moiety in their structures because the aminothiazole moiety interacts with glucokinase (GK) and shows strong GK activation. During the development of *N*-aminothiazol-2-yl amide derivatives, we identified a bioactivation and metabolic liability of 2-aminothizole substructure of GKA **3** by assessing covalent binding, metabolites in liver microsomes and glutathione (GSH) trap assay.

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GK, a member of the hexokinase family, is expressed in the liver and in pancreatic β-cells.[1] This enzyme catalyzes the key initial step for glucose metabolism, that is, phosphorylation of glucose to glucose 6-phosphate. In the liver, GK promotes glycogen synthesis, whilst it enhances insulin secretion from pancreatic β-cells.[2–4] Therefore, GKAs can be expected to function as an anti-hyperglycemic drug, by both increasing glucose uptake in the liver and the potentiation of insulin secretion from pancreatic β-cells.[5–8] As a result of promising preclinical data, many pharmaceutical companies have actively pursued this target aiming at the development of GKAs.[9–19] Of these, several companies including Roche, Astra-Zeneca, and OSI/Prosidion have advanced into clinical studies.[10,12]

Most of the GKAs reported previously possess the *N*-aminothiazol-2-yl amide moiety in their structures because the aminothiazole substructure interacts with Arg63 of GK and shows strong GK activation.[20] We have also developed and reported 2-aminothiazole-2-yl-containing benzamides as GKAs exemplified by compounds **1–3** (Fig. 1).[13–15] Compound **3**, for instance, showed potent GK activation and glucose lowering effects in diabetic mice models.[15] Thus, the GKA **3** was evaluated further to establish its potential for biochemical activation and its metabolic profiles.

As a part of drug discovery and development, the metabolic profiles of new drug candidates must be characterized. Drug metabolites are the products of enzymatic modifications such as oxidation or conjugate formation. Intermediates in the metabolic reactions, or the products themselves, may be reactive and give rise to covalent protein binding. Evaluation of this is important since covalent protein modification may lead to unwanted toxicities and idiosyncratic reactions in human.[21]

In brief, the potential of drug candidates to cause covalent binding is first evaluated in vitro by incubation of a radiolabeled analog in the presence of rat and human liver microsomes under oxidative conditions. In both cases, formation of covalent adducts with protein is determined by successive washing of protein pellets using either Brandel harvester technique or centrifugation-based methods.[22–24] A target value of 50 pmol-equiv/mg protein at 1 h for the in vitro assay was proposed by Evans et al., considering these

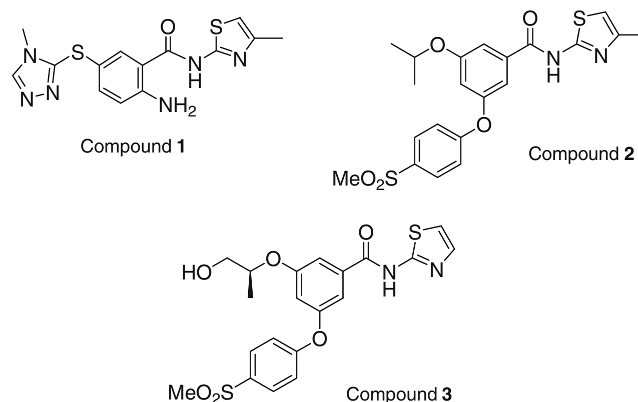

Figure 1. Structures of GK activators **1–3**.

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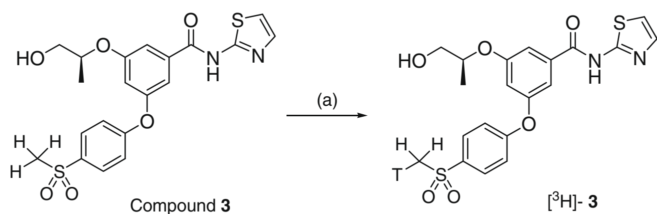


Scheme 1. Preparing of tritium labeled **3**. Reagents and conditions: (a) (1) T_2O–H_2O (5 Ci/mL), DBU, THF, specific activity: 102.2 mCi/mmol, purity: >99.4%.

values are approximately 10-fold over the background of the assays and represent 1/20th of binding obtained for known hepatotoxins.[21]

Table 1
Covalent binding data of compound **3** in human and rat liver microsomes

NADPH	Covalent binding[a]	
	Human MS (HM)	Rat MS (RM)
(+)	715 ± 2	378 ± 23
(−)	10 + 9	0 + 0

[a] Values reported as pmol-equiv binding/mg protein at 1 h.

This paper herein describes the in vitro covalent binding results that were observed during the course of evaluating a GKA **3** for the treatment of type-2 diabetes. Work was first performed to identify what reactive species was responsible for protein labeling, using a glutathione (GSH) as trapping agent.

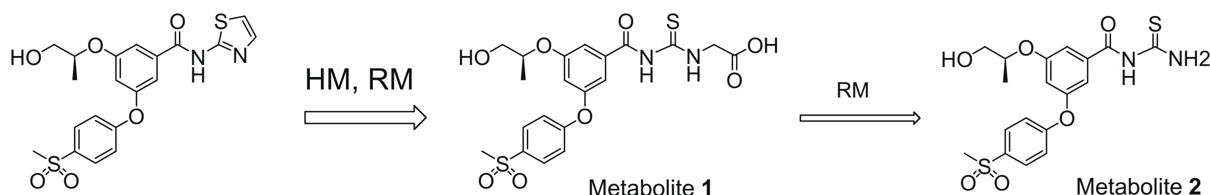
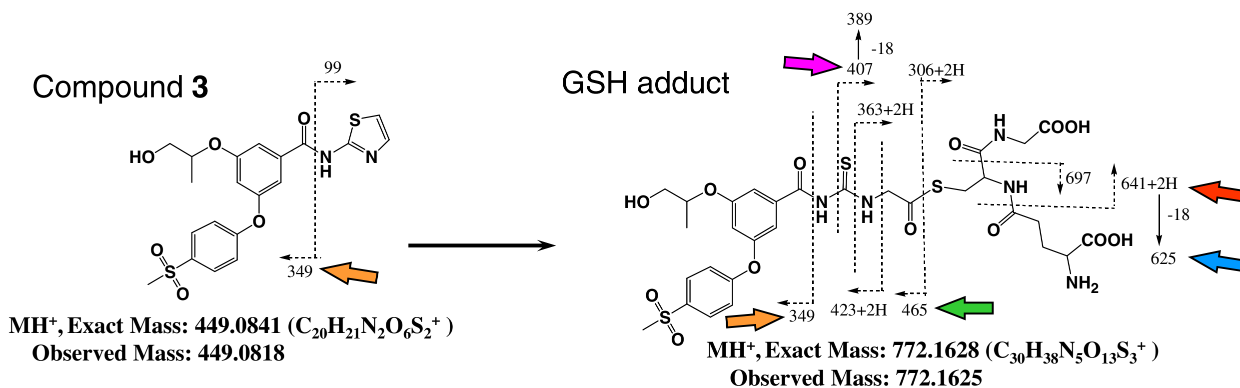


Figure 2. Major metabolites of **3** in human and rat microsomes. HM: Human microsomes, RM: Rat microsomes.



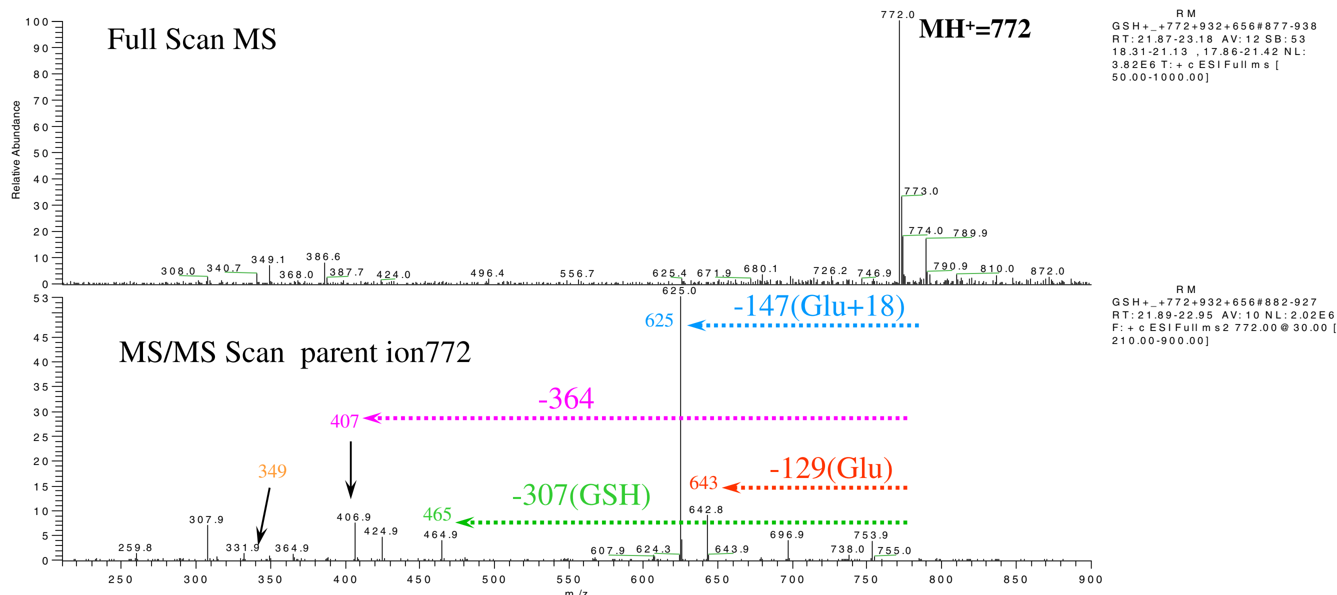


Figure 3. Chemical structure of GSH adduct of **3** in rat microsomes.

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