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Design and synthesis of 2-N-substituted indazolone derivatives as non-carboxylic acid glycogen synthase activators

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ABSTRACT

Glycogen synthase (GS) catalyzes the transfer of glucose residues from UDP-glucose to a glycogen polymer chain, a critical step for glucose storage. Patients with type 2 diabetes normally exhibit low glycogen levels and decreased muscle glucose uptake is the major defect in whole body glucose disposal. Therefore, activating GS may provide a potential approach for the treatment of type 2 diabetes. In order to identify non-carboxylic acids GS activators, we designed and synthesized a series of 2-*N*-alkyl- and 2-*N*-aryl-indazolone derivatives and studied their activity in activating human GS.

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Glycogen synthase (GS) is one of the key regulatory enzymes in glycogen synthesis. It catalyzes the addition of glucose from UDP-glucose to the growing glycogen chain through a 1,4-α linkage and this step is the rate-limiting step for glycogen synthesis under most circumstances.[1,2] GS is regulated covalently through multiple phosphorylation sites and allosterically activated by glucose-6-phosphate (G6P).[3] Two forms of GS exist in mammals, encoded by the genes *GYS1* and *GYS2*. The gene products share 69% homology, where *GYS2* encodes the liver specific form and *GYS1* encodes the form expressed in all other tissues including muscle.[2] By converting excess glucose into part of the glycogen polymer chain, GS plays a key role in glucose storage.

There is genetic and clinical evidence implicating GS in type 2 diabetes (T2D). It has been reported that a polymorphism of the muscle gene *GYS1* is more prevalent in T2D patients than in non-diabetic controls.[4] Both basal and insulin-stimulated GS activity in muscle cells from diabetic subjects are significantly decreased relative to cells from non-diabetic subjects.[5]

The exact molecular mechanism that underlies the decreased glycogen synthesis in T2D is still under investigation and debate. On one side, the use of quantitative ^{13}C NMR spectroscopy in hyperglycemic–hyperinsulinemic clamp studies with ^{13}C-glucose revealed a greater than 50% decrease in muscle glycogen synthesis in NIDDM compared to normal subjects. This decrease paralleled the decrease in glucose uptake and accounted for essentially the entire defect in whole-body glucose disposal.[6] Using the same analysis, it was concluded that decreased muscle glycogen synthesis in diabetic subjects was primarily due to impaired glucose transport.[7] On the other hand, in animal models, muscle-specific disruption of GSK3β (which phosphorylates and inactivates GS) led to improved glucose tolerance, a twofold increase in glycogen accumulation, and an increase in GS activation by insulin without any effect on glucose transport.[8] Therefore, it has been suggested that both glucose transport and GS activity can contribute to the rate of glycogen accumulation, possibly to different extents that may vary under different circumstances.[2,9]

In our metabolic disease research, we were interested in identifying small molecule GS activators. High-throughput screening of our compound library provided a single hit **1** illustrated in Figure 1 (GYS1 EC_{50} = 15.1 μM, 5.8-fold increase at 75 μM).[10] Further hit expansion and lead optimization led to very potent GS activators as exemplified by **2** (GYS1 EC_{50} = 0.14 μM, 5.4-fold increase at 75 μM) and **3** (GYS1 EC_{50} = 0.1 μM, 6.8-fold increase at 75 μM).[11–14] However, these carboxylic acids displayed very low exposure in muscle relative to that in liver after oral dosing in

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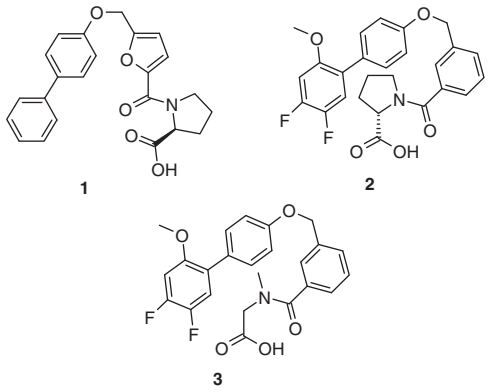


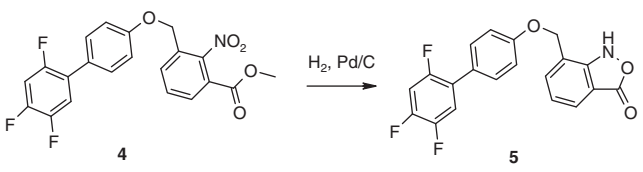
Figure 1. Structures of biphenyl ether derived carboxylic acids as GS activators.

mouse. Therefore, our goal was to identify non-carboxylic acid GS activators with a potential of improved muscle distribution. In this Letter, we report the first non-carboxylic acid GS activator from the 2-N-substituted-indazolone chemical class.

Since the major defect of glucose disposal in T2D is due to impaired glucose incorporation into glycogen in muscles (as shown by the clamp study described in the introduction), compounds with higher muscle exposure could achieve a higher on-target effect by increasing muscle GS activity. To identify non-carboxylic acid GS activators to increase potential muscle exposure, we first investigated derivatives of **3**. Esterification of the carboxylic acid to its corresponding ester abolished GS activation potency. When the carboxylic acid was reduced to a hydroxyl group, significant loss of potency was observed (EC_{50} = 4.1 μM, 7.3-fold increase at 75 μM). Replacing the COOH group in **3** with an ethylenediol also led to large reduction of potency (EC_{50} = 10.3 μM, 6.6-fold increase at 75 μM). We next decided to replace the right hand phenylcarboxamide fragment with heterocycles. One of the designed heterocycles required an intermediate of *ortho*-aminobenzoic acid ester. During the hydrogenation of intermediate **4** (reduction of the nitro group in **4** to an amine, Scheme 1), benzisoxazolone **5** was isolated as a side product, probably through partial reduction of the nitro group to a hydroxylamine followed by cyclization. To our surprise, this side product displayed good potency in GS activation (EC_{50} = 1.0 μM, SC_{200} = 0.41 μM, 4.0-fold increase at 75 μM). However, when **5** was orally dosed to C57 mice, complete breakdown of the bicyclic molecule into *ortho*-aminobenzoic acid was observed, with the parent compound not detected in the plasma.

To resolve the metabolic stability problem in **5**, we explored different bioisosteres to replace the benzisoxazolone. As shown in Figure 2, a series of indazolones was designed and investigated as non-carboxylic acid GS activators.

We initially investigated the application of Buchwald–Hartwig palladium- and copper-catalyzed aromatic amination for the formation of the C–N bond.[15,16] Although it has been reported that copper(I)-catalyzed intramolecular N-arylation of 2-halobenzhydrazide provided an efficient synthesis of 1-N-substituted indazolones,[17] a general and efficient method is still required for the synthesis of 2-*N*-alkyl- and 2-*N*-aryl indazolones such as **6**. We



Scheme 1. Partial reduction of nitro group to form benzisoxazolone.

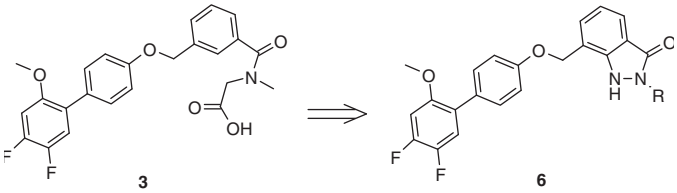


Figure 2. Design of 2-N-substituted indazolones as non-carboxylic acid GS activators.

envisioned the synthesis of **6** through an intermolecular N-arylation reaction between *N*-Boc-*N*-alkylhydrazine or *N*-Boc-*N*-arylhydrazine **7** and the ester of *ortho*-bromobenzoic acid **8** followed by ring closure under acidic conditions as shown in Scheme 2 for the preparation of **10ah** through **10ok**. While the intermolecular N-arylation of *N*-arylhydrazine with aryl halide has been reported using palladium acetate and *tri-tert*-butylphosphonium tetrafluoroborate,[18] the synthetic method we describe here has not been reported in the literature for the preparation of 2-N-substituted indazolones. The required *N*-aryl-*N*-Boc-hydrazines **7d** and **7e** were synthesized according to the literature method through the N-arylation of *N*-Boc-hydrazine with an aryl iodide.[19] The necessary *N*-Boc-*N*-alkylhydrazine was prepared from an alkyl chloride and hydrazine followed by protection of the secondary amine rather than the primary amine.[20] As described in Scheme 3 for the preparation of **7c**, **7f** and **7g**, we observed selective *N*-Boc formation at the alkyl-substituted nitrogen of the hydrazine derivative **11**. The preparation of aryl halides **8h** through **8k** is also presented in Scheme 3. For the synthesis of **10mh** (Table 2), 2,2-diethoxyethylhydrazine was used and the aldehyde group in the indazolone was reduced by sodium borohydride to provide the corresponding alcohol **10mh** (reaction not shown in the scheme).[21]

As shown in Scheme 2, this synthetic sequence made it possible to install different functional groups at the 2-N-position. Selected reaction yields for step (a) and step (b) in Scheme 2 are listed in Table 1. The N-arylation reaction gave decent yields despite the *di-ortho*-substitution pattern. The deprotection of the Boc-group and the subsequent cyclization was completed in one pot with aqueous hydrochloric acid and THF. This method also made it possible for one-pot deprotection of other acid-labile protective groups, such as ester in **9ch** and acetonide in **9fh**, during the indazolone ring formation step (**10lh** and **10nh**).[21] The relatively low yield for **10lh** was due to partial ester hydrolysis under these conditions.

Compounds **10ah** through **10ok** synthesized according to Scheme 2 were studied for their activity as activators of human muscle glycogen synthase (GYS1). The recombinant human muscle GYS1 expressed and partially purified from sf9 cells was used as the glycosyl transferase and the assay was carried out by coupling with pyruvate kinase and lactate dehydrogenase.[22] The more detailed enzyme assay procedure is described in the Supplementary data. The activation potencies are listed in Table 2.

Our earlier work on GS activators was primarily focused on carboxylic acids and acid mimetics.[11–14,23] Although those compounds demonstrated good potency in activating muscle glycogen synthase, they have high protein binding and are mainly distributed in liver when orally dosed in mice. Our rationale to identify non-carboxylic acid GS activators was based on the assumption that neutral compounds might have decreased protein binding and improved muscle distribution relative to the liver. We designed the 2-N-substituted indazolone series to improve the metabolic stability of **5**. As shown in Table 2, all compounds demonstrated GS activation (2.5- to 6.8-fold increase at 75 μM, the highest concentration tested). Results from the dose–response assay indicated a range of EC_{50} (0.2–4 μM) and SC_{200} (0.06–2.75 μM) value. Compound

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