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Synthesis and structure–activity relationships of 3-phenyl-2-propenamides as inhibitors of glycogen phosphorylase *a*

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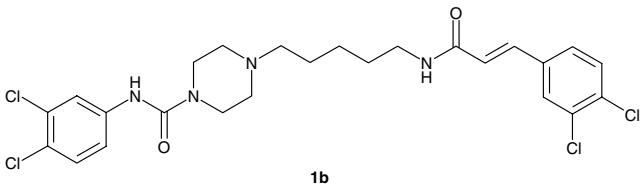
Abstract—A series of 3-phenyl-2-propenamides discovered from a high-throughput screening campaign as novel, potent, glucose-sensitive inhibitors of human liver glycogen phosphorylase *a* is described. A solid-phase synthesis on DMHB resin was also developed which provided efficient access not only to certain analogues that could not be cleanly made using more traditional means, but also to a variety of additional analogues. The SAR scope and synthetic strategy are presented herein.

Type 2 diabetes (non-insulin dependent diabetes mellitus) is a disorder characterized by hyperglycemia. According to the American Diabetes Association (ADA), nearly 16 million Americans have type 2 diabetes. Studies have shown that excessive hepatic glucose production (HGP) is a significant factor contributing to diabetic hyperglycemia.[1] Glucose is produced by both gluconeogenesis and glycogenolysis, the release of glucose-1-phosphate from glycogen. Since glycogenolysis is a major component of HGP and human liver glycogen phosphorylase *a* (GPa) catalyzes this reaction, it is thought that inhibiting GPa will limit glycogenolysis, reduce HGP, and thus lower blood glucose, thereby providing a potential new treatment for type 2 diabetes. In addition, there are several examples in the literature of small molecule allosteric inhibitors of this enzyme.[2] Herein are presented the discovery, synthesis, and structure–activity relationships of a series of 3-phenyl-2-propenamides as novel glucose-sensitive GPa inhibitors.

Compound **1b** was discovered as part of a high-throughput screen of the GSK proprietary compound collection. This compound (**1b**) was found to have both good enzyme inhibition (IC_{50} = 0.94 μM) as well as glucose-sensitivity, being completely devoid of enzyme activity in the absence of glucose (−glu).[3] Clearly, significant metabolic advantages should exist for glucose-sensitive GPa inhibitors which act primarily at the high blood glucose levels typically seen in diabetic patients, and then lose potency as glucose levels fall in order to avoid hypoglycemia.



1b
IC_{50} (+glu) = 0.94 μM
IC_{50} (-glu) > 20 μM

Initial objectives for this chemical series were to develop efficient synthetic methods, increase the potency, and establish key structure-activity relationships. In the initial synthesis of analogues **1**, key diamine intermediates **5** were prepared individually via traditional solution-phase synthesis (Scheme 1). Cinnamic acid **2** was converted to the acid chloride and then coupled to the desired aminoalcohol. Treatment of the alcohol **3** with

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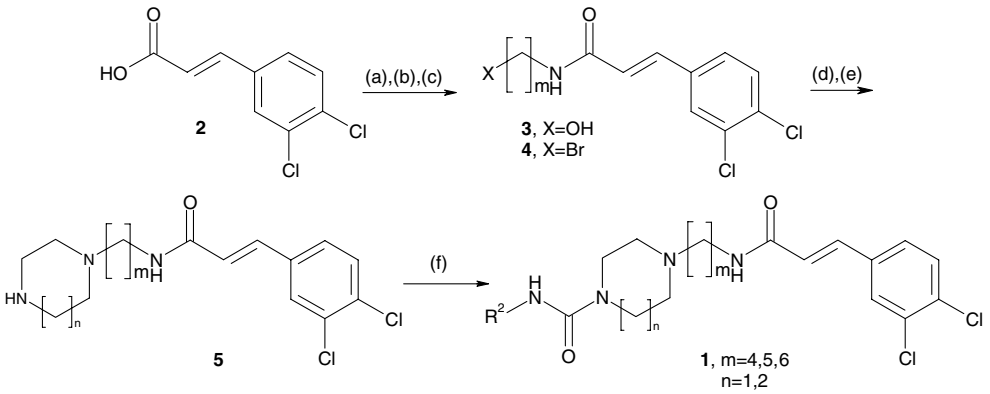
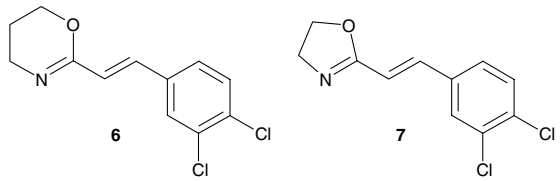


Scheme 1. Reagents and conditions: (a) $(COCl)_2$, 1.5 equiv, DMF, cat., DCM, rt, 2 h; (b) $NH_2(CH_2)_mOH$, 2 equiv, DIPEA, 2 equiv, DCM, 0 °C to rt, 30 min; (c) CBr_4, 1.2 equiv, PPh_3, 1.2 equiv, DCM, rt, 5 h; (d) Boc-piperazine (n = 1), 1 equiv CH_3CN, refl, 6 h; (e) 50% TFA/DCM, 0 °C, 2 h; (f) R^2NCO, 10% TEA/DCM, rt, 30 min.

carbon tetrabromide/triphenylphosphine to form **4**, followed by displacement of the bromide with Boc-piperazine, or Boc-homopiperazine, afforded the key final intermediates after deprotection, in multi-gram quantity. Rapid diversification of the secondary amines (**5**, m = 4–6) with a variety of isocyanates to form the final products **1** was then accomplished in parallel using the Robbins Flex-Chem System.[4] Intermediates **5** could also be reacted with isothiocyanates, sulfonyl chlorides, chloroformates, and acid chlorides in a similar fashion.

Preparation of intermediates **5** (m = 2, 3) was found to be problematic however. For example, when alcohol **3** (m = 3) was subjected to the deoxybromination conditions described in Scheme 1, oxazine **6** was formed as the major product via an intramolecular cyclization. Attempts to convert the alcohol **3** (m = 3) to a tosylate also provided predominantly oxazine **6**. Interestingly, bromide **4** (m = 2) could be formed successfully, but when treated with the cyclic amine still afforded predominantly the oxazoline **7**.[5]

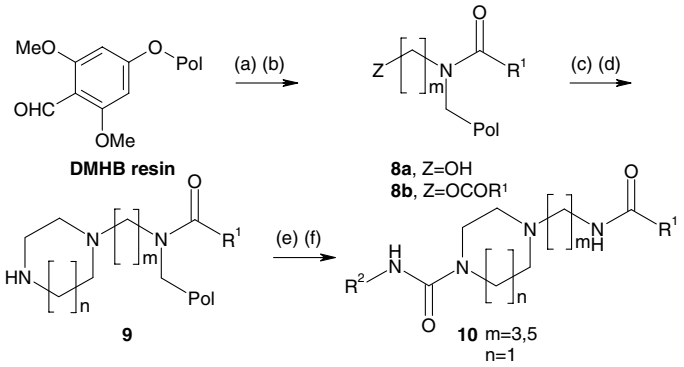


A solid-phase synthesis strategy was conceived to not only address this undesirable side reaction, but to also provide a more efficient and robust method for rapid SAR identification for this series. This solid-phase strategy, which allowed swift diversification of four regions of this molecule, is outlined in Scheme 2.[6]

The aminoalcohols were loaded onto 2-(3,5-dimethoxy-4-formylphenoxy)ethyl polystyrene resin[7] via reductive alkylation. The resin-bound secondary amines were then coupled with the carboxylic acid R^1 using diisopropylcarbodiimide in *N*-methylpyrrolidinone. This reaction was very successful for phenylpropenoic acids (for example, R^1 = 3,4-dichlorophenylpropenoic acid) and provided the desired product **8a** exclusively. Benzoic acids (3,4-dichloro and 4-chlorobenzoic acid) gave primarily the desired intermediates **8a** as well as 15–20% of the bisacylated product **8b**. In contrast, the alkanoic acids (for example, 3,4-dichlorophenylacetic acid), provided only the undesired bisacylated product **8b**. Fortunately, in this case, the resin-bound ester **8b** could simply be treated with base to liberate the primary alcohol **8a** before proceeding to the next step.

The resin-bound alcohol **8a** was next converted to the tosylate, which was directly displaced with an unprotected diamine to afford resin-bound amine **9**. It is noteworthy that no bisalkylated products derived from crosslinking events on the resin were observed when using the unprotected piperazine in the tosylate displacement step under these conditions. The importance of diamine concentration in avoiding crosslinking when using unprotected diamines has been described.[8] More importantly, none of the undesired cyclized byproduct **6** (m = 3) was observed under these conditions. This is presumably due to the fact that in contrast to the solution-phase approach, the amide is now tertiary, with the solid support acting as a protecting group. The resin-bound piperazine **9** was then reacted with a variety of isocyanates to form the corresponding ureas **10** after resin cleavage. Similar conditions were also used successfully to react the amine **9** with acid chlorides, sulfonyl chlorides, and isothiocyanates to explore additional SAR. Lastly, the products were cleaved from the resin using 50% trifluoroacetic acid in dichloromethane. This synthesis was successfully applied in Irori minikan format to produce focused arrays of up to 96 members (m = 3,5; n = 1), with purities of 80–96% by LC–MS (UV 214 nm detection).[9] All compounds were purified by reverse-phase HPLC.[10] In addition, this approach is highly applicable to the preparation of other 3-phenyl-2-propenamide derivatives, which are known in the literature as antiallergic agents,[11] hypotensives,[12] CCR2 antagonists,[13] and dopamine receptor ligands.[14]

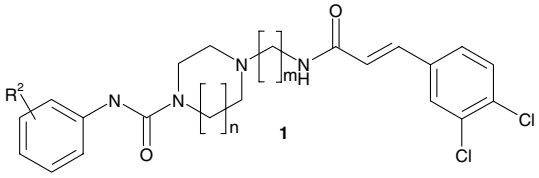
With efficient synthetic methods thus established, the structure–activity relationships of GPa inhibition were explored in four key areas: the urea moiety, the cyclic diamine, the flexible spacer, and the phenylpropenamide portion. Initially, modifications to the urea were exam-



Scheme 2. Reagents and conditions: (a) $NH_2(CH_2)_mOH$, 5 equiv, $NaBH_3CN$, 10 equiv, DCM, rt; (b) 0.33 M R^1CO_2H, 0.4 M 1,3-diisopropylcarbodiimide, NMP, rt, 20 h; (c) 0.33 M *p*-toluenesulfonyl chloride, 0.43 M pyridine, DCM, rt, 20 h; (d) piperazine ($n = 1$), 35 equiv, saturated solution in NMP, 80 °C, 20 h; (e) 0.4 M R^2NCO, NMP, rt, 20 h; (f) 50% TFA/DCM, rt, 2 h.

ined. It was found that compounds in which the phenyl urea was replaced with the corresponding phenyl amide, sulfonamide, or thiourea were inactive ($IC_{50} > 20$ μM). Likewise, replacement of the aryl group on the urea with heteroaryl, hydrogen, or diverse alkyl groups, such as benzyl, branched alkyl, or heteroalkyl, also produced inactive compounds. However, a variety of substitutions on the urea phenyl ring were tolerated, with electron withdrawing groups (**1a**, 4-F, 3-Cl, $IC_{50} = 0.98$ μM, Table 1, or **1b**, 3,4-diCl, $IC_{50} = 0.94$ μM) clearly preferred over electron-donating groups (**1f**, 3,4-OCH_2O, $IC_{50} > 20$ μM). Interestingly, the 3,4-dichloro substituent combination in this series was strongly preferred over the 3-chloro (**1d**) or 4-chloro (**1e**) substituents alone. Additionally, homopiperazine conferred essentially no potency advantage over piperazine when the flexible spacer was a five-carbon chain (**1b**, $IC_{50} = 940$ nM vs. **1j**, $IC_{50} = 470$ nM).

Table 1. In vitro GPa inhibition: urea and diamine variations ($m = 5$)

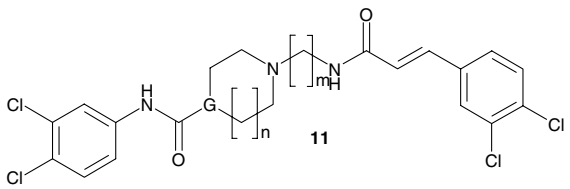


Compound	R^2	*n*	GPa IC_{50} (μM)
1a	4-F,3-Cl	1	0.98
1b	3,4-DiCl	1	0.94
1c	4-Cl, 3-CF_3	1	51
1d	3-Cl	1	51
1e	4-Cl	1	100
1f	3,4-OCH_2O	1	70
1g	3-OMe	1	100
1h	4-Me	1	100
1i	4-F,3-Cl	2	0.72
1j	3,4-DiCl	2	0.47
1k	4-Cl, 3-CF_3	2	1.6
1l	3-Cl	2	1.3
1m	4-Cl	2	51
1n	3-OMe	2	10
1o	4-Me	2	100

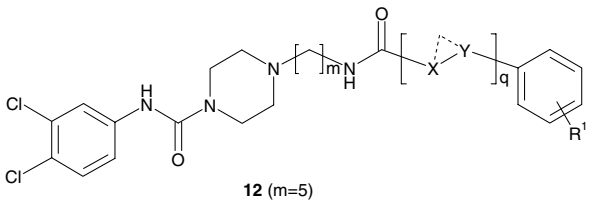
However, the differences between piperazine and homopiperazine were more pronounced when the length of the flexible spacer was varied (Table 2, Compounds **11a**–**h**, **1b**, **1j**) from three to six carbons. For example, an increase in potency with homopiperazine over piperazine was observed when the spacer was shortened to four carbons (**11g**, $IC_{50} = 170$ nM vs. **11c**, $IC_{50} = 710$ nM). Analogues in which there was no carbon spacer or a two-carbon spacer were inactive. Finally, the urea nitrogen of the piperazine ring of **1b** could be replaced with carbon (**11d**, G=C) with no loss in potency.

Variation of the 3-phenylpropenamide moiety was then studied. First, a number of alterations to the double bond were examined, including compounds **12** (R^1 = 3,4-diCl) in which the double bond was replaced with saturated alkyl (X–$Y = CH_2CH_2$), cyclopropyl, and also in which the double bond was removed entirely ($q = 0$). Second, a variety of substituents on the phenyl ring (R^1) were explored. In each case, the resulting compounds were devoid of activity ($IC_{50} > 20$ μM).

Table 2. In vitro GPa inhibition: flexible spacer variations



Compound	*m*	*n*	G	GPa IC_{50} (μM)
11a	2	1	N	90
11b	3	1	N	0.32
11c	4	1	N	0.71
1b	5	1	N	0.94
11d	5	1	C	0.63
11e	6	1	N	0.44
11f	2	2	N	100
11g	4	2	N	0.17
1j	5	2	N	0.47
11h	6	2	N	24



12 (m=5)

The 3,4-dichlorophenylpropenamide was found to be a critical pharmacophore for good potency in this series.

In summary, a series of 3-phenyl-2-propenamides was identified from high-throughput screening as novel, potent, glucose-sensitive inhibitors of human liver glycogen phosphorylase *a*. A solid-phase synthesis on DMHB resin was quickly developed and exploited which not only facilitated rapid SAR generation in four regions, but also provided access to previously problematic analogues. Interestingly, the artificial membrane permeability[15] for compounds in this series ranged from undetectable (**11e**) to moderate (90 nm/s for **11d**). While potent exemplars were identified (**11g**, IC_{50} = 170 nM), this series was not further progressed due to lack of cellular activity (for example, **11c**, IC_{50} (cell) > 10 μM) possibly due to insufficient membrane permeability. A separate series of novel, potent inhibitors with cellular activity has been discovered through high-throughput screening and will be disclosed in a future publication.

Acknowledgments

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3. Inhibitors were tested for human liver glycogen phosphorylase enzymatic activity using a fluorescence intensity endpoint assay. To aid in the identification of glucose-sensitive inhibitors of glycogen phosphorylase, the assay was performed with and without 10 mM glucose. The change in fluorescence due to product formation was measured on a fluorescence plate reader (Viewlux, Perkin-Elmer) using a 525 nm excitation filter and 595 emission filter. The IC_{50} values given are average values of at least two replicates. See WO06/52722 for additional details.
4. The Robbins Flex-Chem system can be purchased from SciGene.
5. Typical product ratio for these reactions was 30% desired product and 70% cyclized by product.
6. Synthesis of related 3-phenyl-2-propenamides via solid-phase using (4-formyl-3-methoxyphenoxy)benzyl polystyrene resin is reported in the literature in Weber, C.; Bielik, A.; Szendrei, G. I.; Keseru, G. M.; Greiner, I. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1279, but without an amine, as in intermediate **5**, for further functionalization.
7. The resin was obtained from Polymer Laboratories, 1.5 mmol/g (PL-FDMP, Product # 1466, 150–300 μm).
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9. All novel compounds were characterized by LC–MS and gave satisfactory results in agreement with the proposed structure. Purity data were determined by a C18 reverse-phase HPLC column [Keystone Aquasil (1 × 40 mm)] in 10–90% ACN/H_2O containing 0.02% TFA (3.6 min gradient) and monitored by a UV detector operating at 214 nm and by a SEDEX 75 evaporative light scattering detector (ELSD) operating at 42 °C. LC–MS M+H signals were consistent with expected molecular weight for all reported products.
10. Preparative HPLC purifications (reverse-phase) were performed using a Gilson with Unipoint software, typically with a YMC 20 × 50 combiprep column (or 30 × 75) ODS-A, 5 μ. A 5-min run (25 mL/min, 10% ACN/H_2O, 0.1%TFA to 90% ACN/H_2O, 0.1% TFA) with UV detection at 254 nm was used.
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15. Compounds were measured in an artificial phospholipid bilayer system using aqueous phosphate buffer, pH 7.05. Compounds of interest are spiked to the donor sides, and after a set time point the samples are withdrawn and analyzed by HPLC with UV detection. The assay detection limit is 3 nm/s.