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# A small molecule antagonist of ghrelin *O*-acyltransferase (GOAT)†

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**Using our recently disclosed fluorescence-based assay to monitor acyltransferase activity, the first non-peptidic, small molecule antagonists of ghrelin *O*-acyltransferase (GOAT), a potential anti-obesity and anti-diabetes target, have been discovered. Each exhibits micromolar inhibition of the enzyme, and may be useful probes for future study of the ghrelin-GOAT system.**

Overweight and obesity currently affect nearly 1 billion people worldwide, and this number is expected to grow in the continuing years.[1] Because of the rising number of cases of overweight and obesity in both adults and children, much effort has been invested in discovering new therapies, both surgical and non-surgical, for these disorders. Although nonsurgical treatments such as drugs are preferred, these efforts have met with limited success. Thus, there is a crucial need to validate new therapeutic targets for the development of effective therapies for overweight and obesity.

Recently, the ghrelin–ghrelin *O*-acyltransferase system has been implicated as a potential target for pharmacologic modulation of obesity.[2,3] Ghrelin is a peptide hormone that acts as a stimulator of growth hormone secretion and is the only circulating peptide shown to potently enhance feeding and weight gain and to regulate energy homeostasis following central and systemic administration.[2] Despite findings suggesting its potential role in overweight and obesity, recent studies have provided evidence that ghrelin acts more as a central neuro-modulator that controls whole-body metabolism, and thus, may be more relevant in diabetic phenotypes.[4,5] Intertwined with ghrelin's intriguing biological activities is also its unique acylated structure, namely a 28-amino acid peptide with an n-octanoylated post-translational modification at Ser-3 (Fig. 1a).[6] Importantly, ghrelin's biological functions are solely dependent on this modification and it is the only known peptide to contain this unique post-translational modification. The enzyme responsible for this acylation, ghrelin *O*-acyltransferase or GOAT, was recently discovered.[7,8] Of significance, murine genetic models for loss or gain of GOAT function have also demonstrated that this enzyme plays an important role in metabolism, similar to ghrelin.[9–11]

To date, only two examples of GOAT antagonists have been reported, and both are peptide-based (Fig. 1b). Compound **1**, discovered by Goldstein and Brown, is based on the ghrelin(1–5) pentapeptide with a diaminopropionic acid residue in place of Ser-3 for added stability of the octanoyl side chain.[12] Only *in vitro* assay data has been published for this compound, and an $IC_{50}$ value of ~1.5 μM was reported.[12] Mechanism-inspired compound **2**, also known as GO-CoA-Tat, was reported by Cole and co-workers, and is based on the premise that GOAT might use a ternary complex mechanism to link octanoyl-CoA and *des*-acyl ghrelin.[11] Both *in vitro* and *in vivo* data have been reported for this compound. Impressively, administration of GO-CoA-Tat in mice was found to reduce weight gain and improve glucose tolerance.[11]

Although these examples provide evidence for the potential impact of GOAT antagonism, the chemical design of compounds **1** and **2** leaves much to be improved upon as both are peptide based. As such, the discovery of more drug-like GOAT antagonists that can be readily synthesized and modified for use as both chemical probes and lead therapeutic candidates against GOAT remains an important goal. Herein, we disclose the first small molecule-based GOAT antagonists. Using our high-throughput screening assay for GOAT, we have examined a small, focused library of compounds and

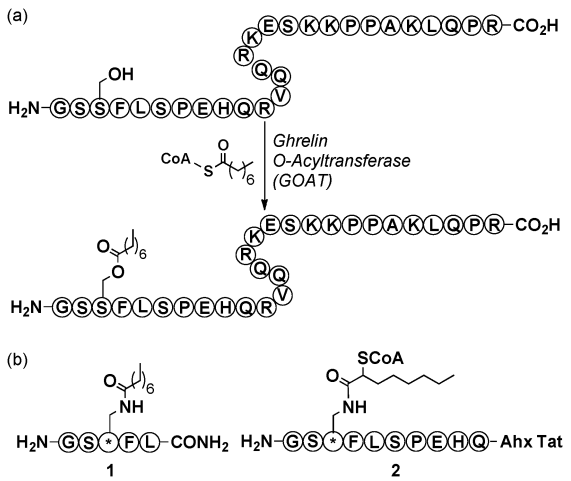


**Fig. 1** (a) GOAT-catalyzed n-octanoyl transfer to ghrelin. (b) Structures of reported GOAT antagonists. * = Diaminopropionic acid. Ahx = aminohexanoate.

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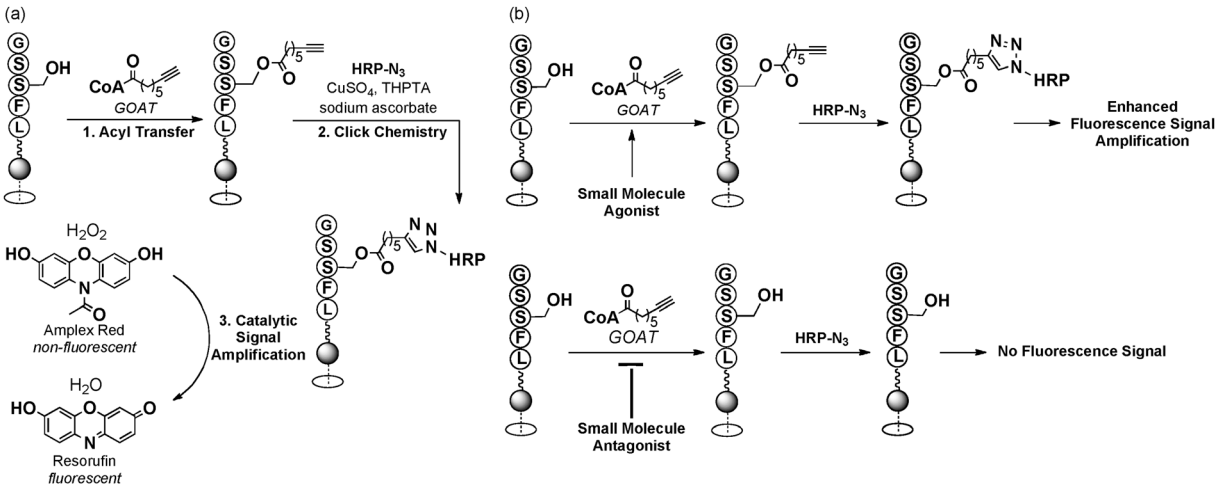


**Fig. 2** cat-ELCCA for screening GOAT activity. (a) General assay approach. (b) Anticipated assay outcome in the presence of GOAT agonists and antagonists. HRP = horseradish peroxidase. THPTA = tris[(3-hydroxypropyl-1*H*-1,2,3-triazol-4-yl)methyl]amine.

have discovered two small molecules with potential as GOAT-targeted probes.

Our group has recently reported a conceptually new enzyme assay based on cat-ELISA, which we have termed catalytic assay using enzyme-linked click chemistry assay, or cat-ELCCA.[13] In cat-ELCCA, an enzyme-linked click chemistry substrate is utilized to arm the assay with catalytic signal amplification necessary for ultrasensitive detection. Using this assay technology, we developed the first fluorescence-based, potentially high-throughput screening system for GOAT (Fig. 2a).[13] The key step of our assay technology involves the use of azido-modified horseradish peroxidase (HRP), which covalently modifies the octynoyl ester of the immobilized ghrelin substrate using click chemistry (see Supporting Information for more detail†). The HRP-triazole product is then detected using amplex red to provide catalytic fluorescence signal amplification of the n-octynoyl ester-HRP triazole product concentration.

Using our cat-ELCCA for GOAT activity design, both agonists and antagonists of GOAT can theoretically be identified (Fig. 2b), which is critical since the exact role of GOAT in obesity and metabolism is currently unknown. Additionally, while most fluorescence-based high-throughput screening assays are subject to compound interference,[14,15] cat-ELCCA does not suffer from such limitations. More specifically, because compound screening and fluorescence measurement are conducted in separate steps, fluorescent compounds will not interfere with the assay read-out since all compounds are removed prior to detection. Thus, cat-ELCCA should provide reproducible and reliable screening results and we were optimistic that our assay could be used to screen for modulators of GOAT activity.

Because we had not previously validated the potential of cat-ELCCA for screening, compound **1** was evaluated as a test compound since an $IC_{50}$ value for this molecule has been reported and it is more synthetically tractable than compound **2**. Varying concentrations of compound **1** were added during the acyl transfer reaction (step 1, Fig. 2a) and the resulting GOAT activity was assayed. Importantly, peptide **1** exhibited dose-dependent inhibition with an $IC_{50}$ value of 1.1 μM, which is in agreement with that reported in the literature (see Supporting Information†).[12] Thus, cat-ELCCA can be used to screen for antagonists of GOAT. However, because no agonists of GOAT activity have been reported, we were unable to validate this potential application.

To target protein–protein interactions, our laboratory proposed the concept of "credit card" libraries.[16,17] More specifically, these libraries are based upon flat, rigid scaffolds elaborated with chemical diversity elements. The underlying concept behind the design of such molecules lies in the fact that the "hot spots" at the interfaces between proteins can be viewed as aromatic, slot-like regions, or "card readers". Thus, a small molecule "credit card" with the correct code or favorable properties to disrupt this space should trigger an event at the protein–protein interface. To demonstrate this concept, a library of compounds based on a naphthalene core was synthesized *via* the Ugi multicomponent reaction, and lead compounds targeting the Myc–Max protein–protein interaction[16] and formation of the HIV-1 gp41 fusogenic core[18] were identified.

Because protein–protein and protein–peptide interactions are also likely to be involved in GOAT-catalyzed n-octanoylation of ghrelin, we were interested in designing a new series of compounds based on our previously reported naphthalene scaffold for modulation of GOAT. Using the Ugi multi-component reaction, a small, focused library of naphthalene-derived compounds (**3**) was synthesized using aliphatic carboxylic acids ($R^1$), varying amines ($R^2$) and benzylisonitrile as diversity elements (Fig. 3a) (see Supporting Information for more detail†). The compounds were then analyzed for agonism or antagonism of GOAT using cat-ELCCA. All compounds were initially examined at 50 μM, and to our delight, two compounds were discovered from this small library as potential GOAT antagonists, compounds **3a** and **3b** (Fig. 3b). No agonists were discovered from this series of compounds. Importantly, upon further examination of compounds **3a** and **3b**, each was found to exhibit dose-dependent antagonism of GOAT with measured $IC_{50}$ values of 7.54 μM and 13.1 μM, respectively (see Supporting Information for inhibition curves†). Thus, using cat-ELCCA we have successfully discovered the first non-peptidic small molecule antagonists of GOAT. It is important to note here that although

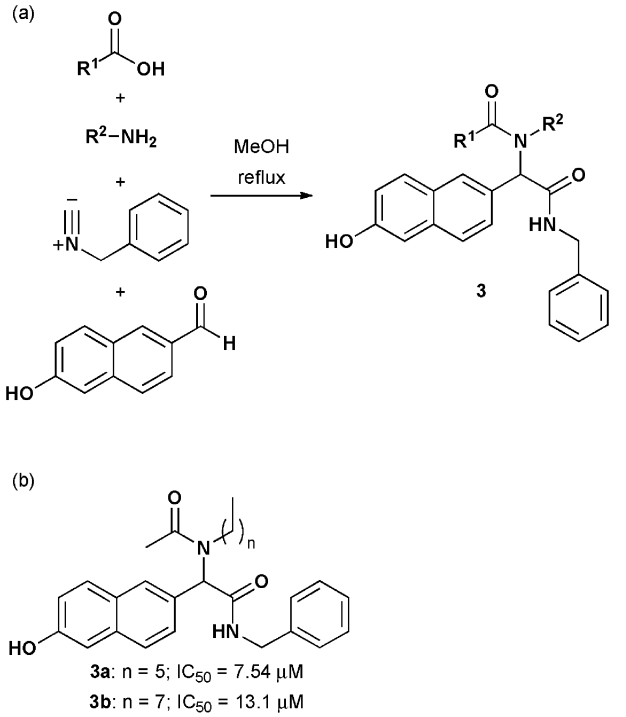


**Fig. 3** (a) Synthesis of potential GOAT agonists or antagonists using the Ugi multicomponent coupling reaction and (b) structures of lead naphthalene-based compounds identified using cat-ELCCA.

these compounds were tested as racemates, one enantiomer may be more potent than the other. Examination of stereochemical impact is currently ongoing.

Based on the structures of compounds **3a** and **3b**, it is plausible to suggest that these compounds block octynoyl-CoA from binding to GOAT since each contains a long alkyl chain similar to that found in octanoyl-CoA. In the cell, acyl-CoA substrates are transported as covalent adducts with acyl-CoA binding proteins. To transfer octanoyl-CoA to ghrelin, GOAT must interact with octanoyl-CoA binding protein, thus, establishing a potentially important protein–protein interaction. Since our crude membrane-bound GOAT extract does not contain octanoyl-CoA binding protein, it will be interesting to examine this possible mechanism in cells. However, it cannot be ruled out that compounds **3a** and **3b** are simply blocking the octanoyl-CoA binding site of GOAT.

In summary, using our recently disclosed fluorescence-based assay for GOAT activity, cat-ELCCA, we have discovered the first non-peptidic, small molecule antagonists of GOAT, a promising anti-obesity and anti-diabetes drug target. Importantly, the identification of such small molecule-based compounds should aid in furthering our understanding of the impact of GOAT modulation in the ghrelin-GOAT system. Although our discovered credit card-based compounds are not the most potent antagonists of GOAT, they are more drug-like than the presently known peptide-based inhibitors. Additionally, based on the ease of chemical synthesis (only 1 step), structure–activity relationship studies may readily be employed to identify more potent GOAT antagonists based on our scaffold. Future cellular-based assay studies and ghrelin-GOAT-based phenotypic studies will be reported in due course.

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