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Citation	Angewandte Chemie - International Edition. 2024, 63(52), p. e202414682
Version Type	VoR
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Development of a FUT8 Inhibitor with Cellular Inhibitory Properties

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Abstract: Core fucosylation is catalyzed by α-1,6-fucosyltransferase (FUT8), which fucosylates the innermost GlcNAc of *N*-glycans. Given the association of FUT8 with various diseases, including cancer, selective FUT8 inhibitors applicable to in vivo or cell-based systems are highly sought-after. Herein, we report the discovery of a compound that selectively inhibits FUT8 in cell-based assays. High-throughput screening revealed a FUT8-inhibiting pharmacophore, and further structural optimization yielded an inhibitor with a K_D value of 49 nM. Notably, this binding occurs only in the presence of GDP (a product of the enzymatic reaction catalyzed by FUT8). Mechanistic studies suggested that this inhibitor generates a highly reactive naphthoquinone methide derivative at the binding site in FUT8, which subsequently reacts with FUT8. Furthermore, prodrug derivatization of this inhibitor improved its stability, enabling suppression of core fucose expression and subsequent EGFR and T-cell signaling in cell-based assays, paving the way for the development of drugs targeting core fucosylation.

Introduction

Glycosylation is a ubiquitous post-translational protein modification (PTM) that plays a crucial role in various biological processes.[1] *N*-Linked glycosylation, which involves the attachment of oligosaccharides to asparagine residues in proteins, is one of the most prominent PTMs and essential for protein folding, protein homeostasis, and modulation of protein–protein interactions.[2] The core fucosylation of *N*-glycans is mediated by α-1,6-fucosyltransferase (FUT8) and involves the transfer of a fucose (Fuc) moiety from GDP-Fuc to the innermost *N*-acetylglucosamine residue of *N*-linked glycans (Figure 1).[3] This reaction is critically significant,[4] as reflected in the high mortality rate of FUT8-knockout mice (ca. 70% after 3 days).[5] Deletion of core fucose from *N*-glycans on IgG antibodies enhances antibody-dependent cellular cytotoxicity (ADCC).[6] Furthermore, core fucosylation regulates the

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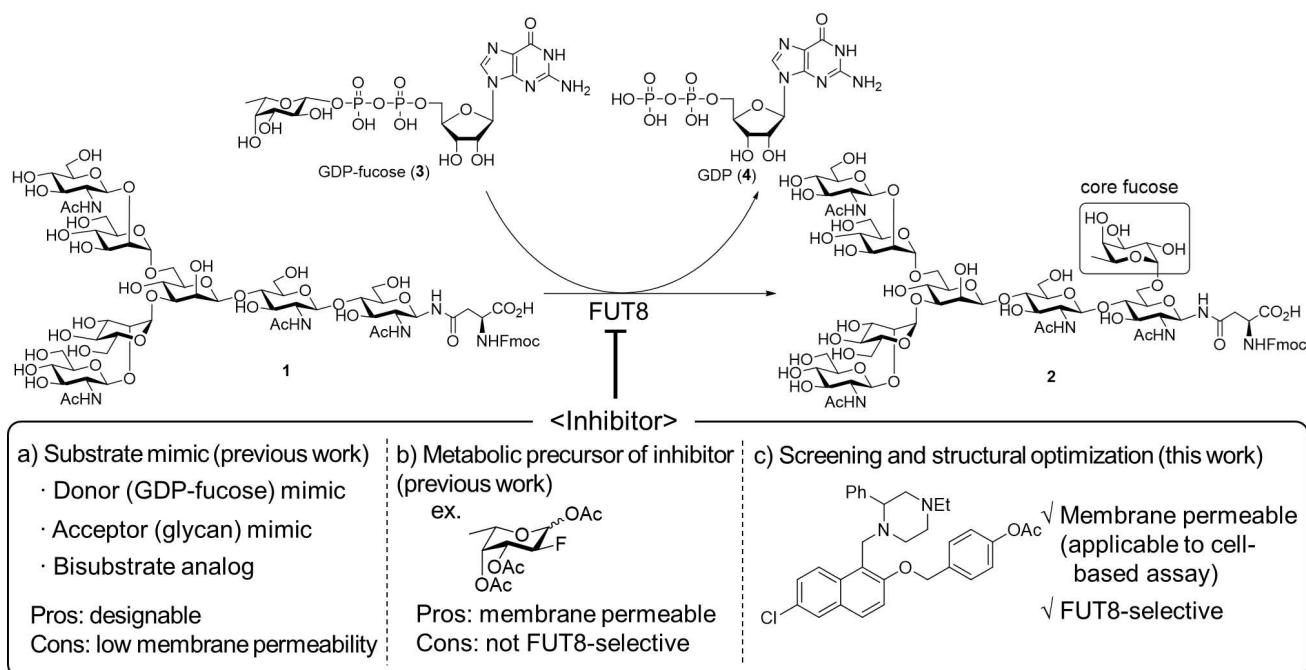1-1 Yamadaoka, Suita, Osaka 565-0871, Japan

Figure 1. FUT8 enzymatic reaction and FUT8 inhibitors. a) Substrate mimic inhibitors. b) Metabolic precursors of inhibitors. c) Inhibitor obtained through screening and structural optimization (this work).

signaling pathways of several growth factors, such as transforming growth factor (TGF),[5] epidermal growth factor (EGF),[7] and vascular endothelial growth factor (VEGF).[8] Core fucose is also closely linked to various diseases; core fucosylated α-fetoprotein (AFP-L3) is a biomarker of hepatocellular carcinoma,[9] and core fucosylation controls tumor metastasis.[10] Inhibiting core fucosylation reduces PD-1 expression on cell surfaces, promotes T-cell activation, and enhances tumor eradication.[11] Moreover, inhibition of core fucosylation attenuates fibrosis[12] and chronic obstructive pulmonary disease (COPD).[13] Core fucosylation of the T-cell receptor (TCR) is essential for T-cell signaling, suggesting a new therapeutic strategy for the treatment of inflammatory bowel disease (IBD) based on inhibiting core fucose formation.[14] Consequently, FUT8 inhibitors are sought-after not only as research tools to explore the biological functions of core fucose, but also as potential lead compounds for advanced treatment strategies for various diseases.

Various glycosyltransferase inhibitors have already been developed, albeit that few are available for in vivo or cellular systems.[15] Most inhibitors mimic the substrates, including donor (generally sugar nucleotides) and acceptor substrates, or both types (bi-substrate mimics). GDP-Fuc mimics have also been reported;[16] however, their low membrane permeability, due to their hydrophilicity, anionic character, and large molecular size, preclude their use in cell-based assays. In contrast, 2-fluorinated peracetyl fucose and 6-alkynyl peracetyl fucose are converted to GDP-Fuc derivatives through metabolic pathways, allowing them to inhibit FUTs in cell-based and in vivo assays (Figure 1b).[17] However, in addition to FUT8, these compounds also inhibit

other FUTs by blocking GDP-fucose biosynthesis, which compromises their efficacy. Recently, Li and Wang et al. have developed the FUT8 inhibitors, which exhibits potent efficacy against metastatic colorectal cancer in vivo.[18]

Here, we report a FUT8-selective inhibitor that functions in cellular systems (Figure 1c), which was developed through a high-throughput screening (HTS) and subsequent structural optimization. Compared to other reported HTS systems, this newly developed HTS system is concise, practical, and reliable.[19] Through screening approximately 33000 compounds, we identified a FUT8 inhibitor pharmacophore. Structural optimization based on this pharmacophore yielded a FUT8 inhibitor with a K_D value of 49 nM. Investigation of the inhibition mechanism revealed that the inhibitor binds to FUT8 in the presence of the FUT8 reaction product GDP. Prodrug derivatization improved the stability of the compound, enabling inhibition of core fucosylation in living cells and regulating EGF signaling and T-cell signaling. This FUT8 inhibitor is a powerful tool for elucidating the role of core fucose and could lead to the development of novel therapeutic approaches that target core fucosylation.

Results and Discussion

High-Throughput Screening (HTS)

We explored FUT8 inhibitors from the compound library of Osaka University (Figure 2 and Figure S1). We first constructed the HTS assay system, in which the GDP produced by the FUT8 enzymatic reaction was quantified using
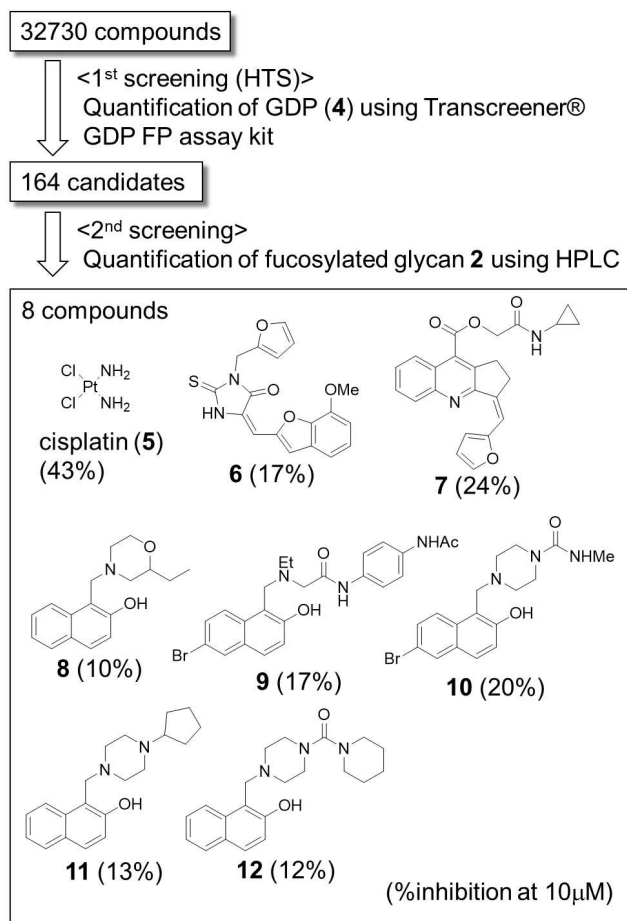
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Figure 2. HTS of FUT8 inhibitors from 32730 compounds. The first screening, in which GDP was quantified using a Transcreener® GDP FP assay kit, gave 164 hit compounds. The second screening, in which the fucosylated *N*-glycan **2** produced by the FUT8 reaction was quantified, identified 8 FUT8 inhibitors. The inhibition rate of each compound at a concentration of 10 µM is shown in parentheses.

fluorescence polarization (FP) with a Transcreener® GDP FP assay kit. When this assay was performed in 360 wells of 384-well microplates (the columns at either end were not used) in the absence of the inhibitor candidates, minimal differences between the wells were observed, and a high Z'-factor was obtained, confirming the reliability of the assay ($Z' = 0.81$; $Z' > 0.7$ is the typical threshold for reliable data). We then screened 32730 compounds, of which 164 were identified as potential FUT8 inhibitors. To eliminate false positives, we performed a second screening, in which the fucosylated *N*-glycan **2** produced by the FUT8 reaction was directly quantified using HPLC. Among the 164 candidates, 8 compounds (Figure 2; **5–12**) inhibited the FUT8 reaction at a concentration of 10 µM. Unexpectedly, cisplatin, a well-known anticancer drug, was identified as a FUT8 inhibitor. The mechanism of FUT8 inhibition by cisplatin is discussed in the Supporting Information (Figure S2). Notably, cisplatin inhibited FUT8 at concentrations lower than those exhibiting cytotoxicity; incubation of PK-8 with 100 µM cisplatin suppressed core fucose expression (Figure S2e) without

displaying cytotoxicity, suggesting that FUT8 may be one of the off-targets of cisplatin. More importantly, five compounds (**8–12**) shared a common 1-methylaminonaphthol structure, which represents a FUT8-inhibitor pharmacophore.

Structural Optimization

We next conducted a structural optimization based on the obtained pharmacophore to develop a more potent inhibitor (Figure 3). To determine the optimal structure efficiently, the pharmacophore was divided into two units: the amine unit and the naphthol unit.

Derivatization of the naphthol unit significantly influenced the FUT8 inhibition activity (Table 1). Different naphthol units were investigated, using morpholine as the amine unit in each case, and the inhibition rates for each compound at concentrations of 10 µM and 100 µM were measured. Deletion or methylation of the phenolic hydroxy group resulted in loss of the inhibition activity (**14** and **15**), indicating that this functional group was essential to the inhibition. Halogen substitution at the 6-position of naphthol enhanced its activity (**16** and **17**), whereas modifications at the 3-position were not tolerated (**18** and **19**). Other derivatizations of the naphthol unit also significantly altered the FUT8 inhibition activity (**20–22**). Based on these results, we selected **17** as the naphthol unit for the final inhibitor.

The structure of the amine unit also affected the inhibition activity (Table 2). The compound with an amine unit without substituents showed no inhibition activity (**23**), whereas mono- and di-benzylation of the amine restored the inhibition (**24** and **25**). Thus, various tertiary amine structures were investigated (**24–32**). All tested compounds inhibited FUT8 at 100 µM, and large substituents were also tolerated. A further structure–activity-relationship (SAR) study of the amine unit based on **28** resulted in **34** as the optimal amine structure (Table 3).

We then designed and synthesized FUT8 inhibitor **37** by combining the naphthol unit of **17** with the amine unit of **34**.
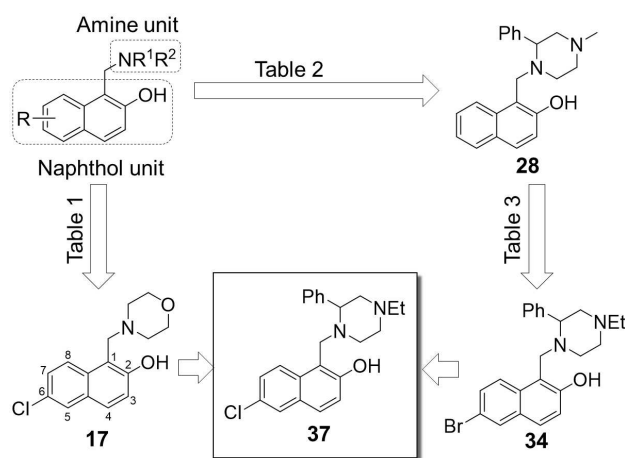

Figure 3. Summary of the structural optimization of the FUT8 inhibitor.

Table 1: SAR study of the naphthol unit.

Compound number	R^1	% inhibition at 10 µM	% inhibition at 100 µM
13		ND[a]	54%
14		ND[a]	ND[a]
15		ND[a]	ND[a]
16		37%	67%
17		39%	70%
18		ND[a]	ND[a]
19		ND[a]	35%
20		ND[a]	12%
21		ND[a]	ND[a]
22		57%	72%

[a] Not detected.

Compound **37** exhibited FUT8 inhibition activity with an IC$_{50}$ value of ca. 10 µM. Both enantiomers of **37** showed almost the same inhibition activity (Table S1).

Inhibition Mechanism

We then investigated the mechanism of the inhibition of FUT8 by **37**. Unexpectedly, **37** was unstable under aqueous conditions (half-life in PBS buffer: $T_{1/2} = 1.1$ h; Figure S6), and the decomposition product was identified as **39** (Figure S3). At this point, we assume that **39** is formed by the addition of water to the exomethylene intermediate **38**,

Table 2: SAR study of the amine unit (1).

Compound number	NR^{2}R^3	% inhibition at 10 µM	% inhibition at 100 µM
23		ND[a]	ND[a]
24		ND[a]	18%
25		ND[a]	23%
26		ND[a]	10%
27		12%	61%
28		23%	78%
29		28%	39%
30		ND[a]	15%
31		18%	53%
32		14%	79%

[a] Not detected.

Table 3: SAR study of the amine unit (2).

Compound number	R^4	% inhibition at 10 µM
33	Me	27%
34	Et	56%
35		28%
36		36%

which can be generated by the elimination of the amine unit from **37** (Figure 4a). These results evoked the hypothesis that **37** could inhibit FUT8 by forming a covalent bond with FUT8 after generating **38**. This hypothesis was confirmed by LC–MS analysis: after treating FUT8 with **37**, adducts with **38** were detected by LC–MS (Figure S4). A similar covalent inhibitory mechanism, involving the formation of quinone methide followed by the formation of a covalent bond through nucleophilic addition, has previously been observed,[20] and unexpected reversible inhibition properties have been reported.[20c] Indeed, after treating **37** at a sufficient concentration to react with all FUT8, the removal of **37** led to partial restoration of the enzymatic activity of FUT8 (Figure S5a). Although thiols are typically considered the primary targets of quinone methides, all eight Cys residues in FUT8 form disulfide bonds, suggesting that other nucleophiles, such as amino groups, may be involved in the reaction. Unfortunately, due to this reversibility, the identification of the binding sites of the inhibitor was not successful. In isothermal-titration-calorimetry (ITC) experiments, no binding was observed in the absence of GDP, while the K_D value between **37** and FUT8 was estimated to be 49 nM in the presence of GDP (Figure 4b and Table S2), suggesting that **37** might bind around the GDP-fucose binding site. Alternatively, given that GDP binding significantly alters the conformation of FUT8,[21] **37** might specifically bind to this altered form of FUT8. Additionally, such high affinity strongly indicates that recognition of **37** by FUT8 occurs prior to the generation of **38**. Furthermore, hydrolyzed **39** exhibited inhibitory activity, albeit that this activity was significantly weaker than that of **37** (Fig-

ure S5b), which also suggests that **37** is preferentially recognized by FUT8. Overall, the following mechanism was revealed for the inhibition of FUT8 by **37**: after **37** is recognized by FUT8 with high affinity, the highly reactive **38** is generated and reacts with FUT8 to inhibit its activity.

Compound **37** binds covalently to FUT8 and inhibits its activity, which represents a new approach to covalent drug design. Covalent drugs have recently gained attention as a modality that can render previously undruggable targets druggable due to their potent and persistent effects. Typically, covalent drugs contain electrophilic functional groups, such as Michel acceptors, epoxides, and haloacetamides. Fine-tuning the reactivity of these electrophiles is essential to reduce off-target effects and achieve high target selectivity.[22] In contrast, **37** is inherently unreactive and is assumed to generate the highly reactive electrophile **38** at the FUT8 binding site to form a covalent bond. When **38** is generated outside the binding site, it is expected to be quenched through reaction with water due to its high reactivity. Indeed, when **37** was incubated with 1 mM of various nucleophilic amino acids (Arg, Asn, His, Lys, Met, Ser, Thr, Trp, Tyr), only water adduct **39** was observed in most cases (a trace amount of the His adduct was also produced; cf. Figure S7). In contrast, in the presence of glutathione (1 mM), the glutathione adduct was the primary one obtained, suggesting that **38** is more reactive toward thiols than other nucleophiles. The present two-step covalent inhibition mechanism, which consists of ligand recognition followed by reaction with a highly reactive species generated at the binding site, represents a new covalent drug design methodology that exhibits high selectivity. Histone deacetylase (HDAC) inhibitors based on a similar mechanism, in which Zn chelation acts as a trigger to generate reactive species, have already been reported.[20c] Although no metal is involved in the enzymatic reaction of FUT8,[3] the generation of **38** from **37** might be triggered by the protonation of an amino group through its recognition by FUT8, contributing to the high selectivity. Additionally, the reversibility of the covalent bonding may help reduce off-target effects by facilitating the removal of nonspecifically bound **38**. Overall, a detailed analysis of the interaction between FUT8 and **37** revealed the unique behavior of **37**, suggesting a novel methodology for developing selective covalent drugs.

Figure 4. FUT8-inhibition mechanism of **37**. a) Putative inhibition mechanism of **37**. b) ITC analysis between FUT8 and **37** in the presence/absence of GDP (1 mM).

Prodrug Derivatization and Cell-Based Assay

For use in a cell-based assay, **37** was converted into a prodrug. Due to the rate of glycan metabolism, several days are required to obtain significant results in the bioassays. However, **37** decomposed rapidly in PBS buffer ($T_{1/2} = 1.1$ h). Therefore, we converted **37** into a prodrug to enhance its stability (Figure S6). Its stability was not sufficiently enhanced by acylation of the hydroxy group of naphthol, including acetylation and benzoylation, but was significantly improved by converting it to **40** (Figure 5 and Figure S6; $T_{1/2} > 12$ h), where the acetylated *p*-hydroxybenzyl group is expected to be cleaved by intracellular esterases.
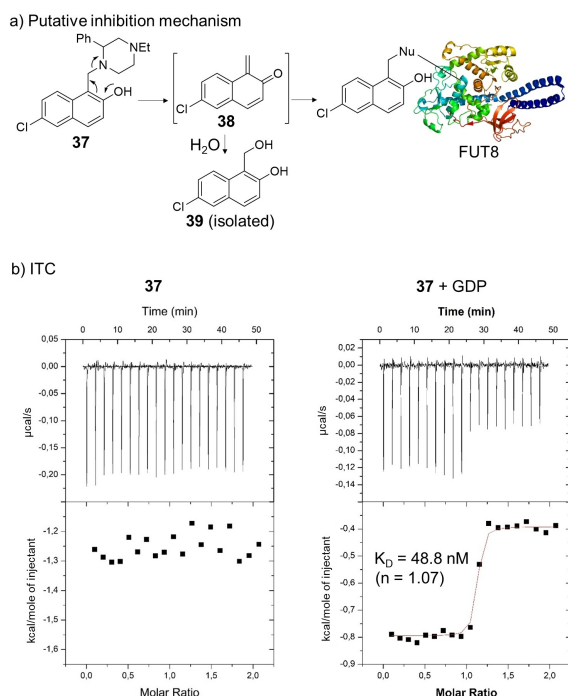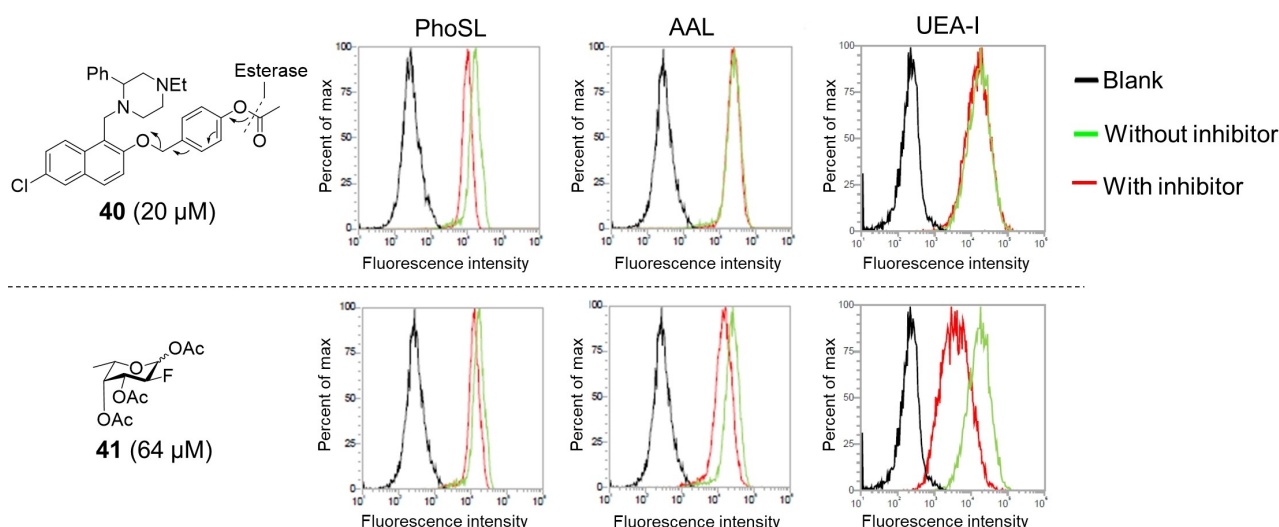
Figure 5. FUT8-inhibition assay in the cell-based assay using flow cytometry. After treatment with the inhibitors (top: **40**, 20 μM; bottom: **41**, 64 μM) for 3 days, the fucosylation levels were evaluated by flow cytometry using PhoSL (α-1,6-fucose (core fucose)-specific lectin), AAL (all α-linked fucose recognition lectin), and UEA-I (α-1,2-fucose-specific lectin).

Compound **40** significantly inhibited FUT8 activity in a cell-based assay (Figure 5). After incubation with **40** (20 μM) for 3 days, the expression levels of fucosylated glycans were evaluated through flow cytometry using PhoSL (α-1,6-fucose (core fucose)-specific lectin), AAL (all α-linked fucose recognition lectin; binding affinity: α-1,6 > α-1,3 > α-1,4 > α-1,2), and UEA-I (α-1,2-fucose-specific lectin). As a positive control, we used 2-fluorinated peracetyl fucose **41** (64 μM), which acts as a universal inhibitor of FUTs.[17b,c, 23] The use of **40** resulted in a larger peak shift in PhoSL detection, whereas **41** caused a peak shift in AAL or UEA-I detection, indicating that **40** selectively inhibits FUT8 without inhibiting other FUTs. Therefore, **40** is a FUT8-selective inhibitor applicable to living cell systems.

We further applied **40** for the regulation of signal transduction. EGF signaling is critical for tumor growth, and the EGF receptor (EGFR) is a well-established molecular target for cancer therapy.[24] Previous FUT8-knockout experiments revealed that the removal of core fucose from EGFR attenuates its signal transduction,[7] suggesting potential for FUT8 inhibitors as anti-cancer agents. Based on these considerations, we examined the effect of **40** on EGFR signaling. After incubation of HepG2 hepatoma cells with **40** (20 μM)/**41** (64 μM) for 3 days, the phosphorylation of EGFR was evaluated (Figure 6 and Figure S8). As expected, **40** significantly suppressed EGFR phosphorylation. In contrast, **41** exhibited less inhibition of phosphorylation, highlighting the effectiveness of selective core-fucosylation inhibition over universal fucosylation inhibition. Furthermore, given that FUT8-knockout suppresses T-cell inflammatory responses,[14] we examined the effect of the FUT8 inhibitor on the suppression of T-cell signaling. As expected, the FUT8 inhibitor significantly suppressed T-cell signaling (Figure S9), indicating a potential use as an immunomodulator. These results demonstrate the potential
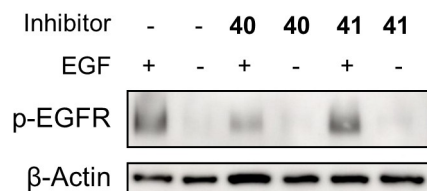

Figure 6. EGF signal inhibition by **40**. After treatment with the inhibitors (**40**: 20 μM; **41**: 64 μM) for 3 days, EGFR phosphorylation was evaluated using western blotting. Quantified data based on band intensity (normalization with β-actin expression level): **40**: 85% inhibition; **41**: 44% inhibition. Uncropped images are shown in the Supporting Information.

of **40** to regulate core fucose functions and, consequently, modulate various biological phenomena.

Conclusion

Core fucosylation regulates various biological phenomena, and thus, FUT8 inhibitors that are applicable in vivo or in cellular systems are highly sought-after. Although several GDP-fucose mimics have been reported as FUT inhibitors, these are unsuitable for living cell systems owing to their low membrane permeability. 2-Fluorinated peracetyl fucose (**41**) inhibits fucosylation in cellular and in vivo systems,[17a] and thus, **41** has been widely used to elucidate and regulate fucose functions. However, **41** lacks FUT selectivity, which compromises its efficacy in FUT8 inhibition. On the other hand, the inhibitor developed in this study selectively inhibits FUT8 in cellular systems. Despite the fact that drugs targeting FUT8 have not yet been developed, FUT8 inhibitors are expected to represent lead compounds for many diseases, including cancer,[10] fibrosis,[12] and COPD.[13] Indeed, FDW028, a recently developed FUT8 inhibitor,

exhibits potent efficacy against metastatic colorectal cancer.[18a] Furthermore, core fucosylation regulates various immune responses, including antibody activity.[6,11] Therefore, FUT8 is an unexplored but promising drug target. Indeed, **40** inhibited EGF signaling in a cell-based assay, demonstrating its potential as an anticancer agent. In vivo experiments using **40** to develop new treatments for several diseases are currently in progress in our group.

We have constructed a practical high-throughput-screening (HTS) system for the exploration of FUT8 inhibitors and demonstrated its usefulness. Our HTS system offers a wide range of potential applications. We detected the nucleotide (GDP) generated from the nucleotide sugar (GDP-fucose) via FP using an anti-GDP antibody. Considering that all glycosyltransferases use nucleotide sugars, including UDP-sugars (α-D-Glc, α-D-Gal, α-D-GalNAc, α-D-GlcNAc, α-D-Xyl), GDP-sugars (α-D-Man, α-D-Fuc), and CMP-β-D-Neu5Ac, and that the antibodies against the respective nucleotides are commercially available, the HTS system developed herein can be expected to be universally applicable for the exploration of inhibitors of all glycosyltransferases.

The well-known anticancer drug cisplatin was identified as a FUT8 inhibitor. While various molecules such as cytoplasmic nucleophiles, glutathione, methionine, metallothionein, and other proteins have been reported as off-targets of cisplatin,[25] no glycosyltransferase off-targets have been previously identified. Core fucosylation enhances the signal transduction of several growth factors, including EGF[7] and VEGF,[8] suggesting that the inhibition of core fucosylation suppresses tumor growth. Therefore, our findings suggest that the anticancer activity of cisplatin may be partially attributed to FUT8 inhibition.

In summary, we have developed FUT8-selective inhibitor applicable to cellular systems. The screening of approximately 33000 compounds led to the identification of a FUT8 inhibitor pharmacophore. Subsequent SAR studies based on this pharmacophore yielded **37** with a K_D value of 49 nM, which was obtained in the presence of GDP. Mechanistic studies revealed that **37** covalently inhibits FUT8. Prodrug derivatization of **37** to yield **40** enhanced its stability, enabling the effective inhibition of FUT8 in cell-based assays. More stable prodrug formulations might enhance the efficacy. Furthermore, designing prodrugs that selectively release **37** in targeted tissues could be advantageous for in vivo applications. Importantly, the developed FUT8 inhibitors achieved the regulation of EGFR and T-cell signaling. These FUT8 inhibitors can be expected to serve as valuable tools for modulating core fucose activity, thereby regulating various biological phenomena.

Supporting Information

The authors have cited additional references within the Supporting Information.[26]

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

[1] a) M. M. Fuster, J. D. Esko, *Nat. Rev. Cancer* **2005**, *5*, 526–542; b) Y. van Kooyk, G. A. Rabinovich, *Nat. Immunol.* **2008**, *9*, 593–601; c) R. G. Lichtenstein, G. A. Rabinovich, *Cell Death Differ.* **2013**, *20*, 976–986; d) C. Reily, T. J. Stewart, M. B. Renfrow, J. Novak, *Nat. Rev. Nephrol.* **2019**, *15*, 346–366.

[2] A. Helenius, M. Aebi, *Science* **2001**, *291*, 2364–2369.

[3] N. Uozumi, S. Yanagidani, E. Miyoshi, Y. Ihara, T. Sakuma, C.-X. Gao, T. Teshima, S. Fujii, T. Shiba, N. Taniguchi, *J. Biol. Chem.* **1996**, *271*, 27810–27817.

[4] M. Takahashi, Y. Kuroki, K. Ohtsubo, N. Taniguchi, *Carbohydr. Res.* **2009**, *344*, 1387–1390.

[5] X. Wang, S. Inoue, J. Gu, E. Miyoshi, K. Noda, W. Li, Y. Mizuno-Horikawa, M. Nakano, M. Asahi, M. Takahashi, N. Uozumi, S. Ihara, S. H. Lee, Y. Ikeda, Y. Yamaguchi, Y. Aze, Y. Tomiyama, J. Fujii, K. Suzuki, A. Kondo, S. D. Shapiro, C. Lopez-Otin, T. Kuwaki, M. Okabe, K. Honke, N. Taniguchi, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 15791–15796.

[6] a) T. Shinkawa, K. Nakamura, N. Yamane, E. Shoji-Hosaka, Y. Kanda, M. Sakurada, K. Uchida, H. Anazawa, M. Satoh, M. Yamasaki, N. Hanai, K. Shitara, *J. Biol. Chem.* **2003**, *278*, 3466–3473; b) R. L. Shields, J. Lai, R. Keck, L. Y. O'Connell, K. Hong, Y. G. Meng, S. H. A. Weikert, L. G. Presta, *J. Biol. Chem.* **2002**, *277*, 26733–26740; c) M. Satoh, K. Shitara, N. Hanai, *Trends Glycosci. Glycotechnol.* **2006**, *18*, 129–136; d) R. Niwa, E. Shoji-Hosaka, M. Sakurada, T. Shinkawa, K. Uchida, K. Nakamura, K. Matsushima, R. Ueda, N. Hanai, K. Shitara, *Cancer Res.* **2004**, *64*, 2127–2133.

[7] X. Wang, J. Gu, H. Ihara, E. Miyoshi, K. Honke, N. Taniguchi, *J. Biol. Chem.* **2006**, *281*, 2572–2577.

[8] X. Wang, T. Fukuda, W. Li, C.-x. Gao, A. Kondo, A. Matsumoto, E. Miyoshi, N. Taniguchi, J. Gu, *J. Biochem.* **2009**, *145*, 643–651.

[9] a) K. Noda, E. Miyoshi, N. Uozumi, S. Yanagidani, Y. Ikeda, C.-x. Gao, K. Suzuki, H. Yoshihara, M. Yoshikawa, K. Kawano, N. Hayashi, M. Hori, N. Taniguchi, *Hepatology* **1998**, *28*, 944–952; b) K. Noda, E. Miyoshi, N. Uozumi, C.-X. Gao, K. Suzuki, N. Hayashi, M. Hori, N. Taniguchi, *Int. J. Cancer* **1998**, *75*, 444–450.

[10] a) K. Moriwaki, K. Noda, Y. Furukawa, K. Ohshima, A. Uchiyama, T. Nakagawa, N. Taniguchi, Y. Daigo, Y. Nakamura, N. Hayashi, E. Miyoshi, *Gastroenterology* **2009**, *137*, 188–198.e2; b) K. Tanaka, K. Moriwaki, S. Yokoi, K. Koyama, E. Miyoshi, K. Fukase, *Bioorg. Med. Chem.* **2013**, *21*, 1074–1077.

[11] M. Okada, S. Chikuma, T. Kondo, S. Hibino, H. Machiyama, T. Yokosuka, M. Nakano, A. Yoshimura, *Cell Rep.* **2017**, *20*, 1017–1028.

[12] a) N. Shen, H. Lin, T. Wu, D. Wang, W. Wang, H. Xie, J. Zhang, Z. Feng, *Kidney Int.*, **84**, 64–77; b) N. Wang, Y. Deng, A. Liu, N. Shen, W. Wang, X. Du, Q. Tang, S. Li, Z. Odeh, T. Wu, H. Lin, *Sci. Rep.* **2017**, *7*, 16914.

[13] C. Gao, T. Maeno, F. Ota, M. Ueno, H. Korekane, S. Takamatsu, K. Shirato, A. Matsumoto, S. Kobayashi, K. Yoshida, S. Kitazume, K. Ohtsubo, T. Betsuyaku, N. Taniguchi, *J. Biol. Chem.* **2012**, *287*, 16699–16708.

[14] H. Fujii, S. Shinzaki, H. Iijima, K. Wakamatsu, C. Iwamoto, T. Sobajima, R. Kuwahara, S. Hiyama, Y. Hayashi, S. Takamatsu, N. Uozumi, Y. Kamada, M. Tsujii, N. Taniguchi, T. Takehara, E. Miyoshi, *Gastroenterology* **2016**, *150*, 1620–1632.

[15] a) T. M. Gloster, *Biochem. Soc. Trans.* **2012**, *40*, 913–928; b) T. M. Gloster, D. J. Vocadlo, *Nat. Chem. Biol.* **2012**, *8*, 683–694; c) T. Kajimoto, M. Node, *Synthesis* **2009**, 3179–3210.

[16] a) Z. Tu, Y.-N. Lin, C.-H. Lin, *Chem. Soc. Rev.* **2013**, *42*, 4459–4475; b) M. D. Burkart, S. P. Vincent, A. Düffels, B. W. Murray, S. V. Ley, C.-H. Wong, *Bioorg. Med. Chem.* **2000**, *8*, 1937–1946; c) B. W. Murray, V. Wittmann, M. D. Burkart, S.-C. Hung, C.-H. Wong, *Biochemistry* **1997**, *36*, 823–831; d) J. I. Luengo, J. G. Gleason, *Tetrahedron Lett.* **1992**, *33*, 6911–6914; e) A. J. Norris, J. P. Whitelegge, M. J. Strouse, K. F. Faull, T. Toyokuni, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 571–573; f) Y. Ichikawa, Y. C. Lin, D. P. Dumas, G. J. Shen, E. Garcia-Junceda, M. A. Williams, R. Bayer, C. Ketcham, L. E. Walker, *J. Am. Chem. Soc.* **1992**, *114*, 9283–9298; g) L. Qiao, B. W. Murray, M. Shimazaki, J. Schultz, C.-H. Wong, *J. Am. Chem. Soc.* **1996**, *118*, 7653–7662; h) C. H. Wong, D. P. Dumas, Y. Ichikawa, K. Koseki, S. J. Danishefsky, B. W. Weston, J. B. Lowe, *J. Am. Chem. Soc.* **1992**, *114*, 7321–7322; i) M. L. Mitchell, F. Tian, L. V. Lee, C.-H. Wong, *Angew. Chem. Int. Ed.* **2002**, *41*, 3041–3044; j) G. Carchon, F. Chrétien, P. Delannoy, A. Verbert, Y. Chapleur, *Tetrahedron Lett.* **2001**, *42*, 8821–8824; k) L. V. Lee, M. L. Mitchell, S.-J. Huang, V. V. Fokin, K. B. Sharpless, C.-H. Wong, *J. Am. Chem. Soc.* **2003**, *125*, 9588–9589; l) Y. Manabe, S. Kasahara, Y. Takakura, X. Yang, S. Takamatsu, Y. Kamada, E. Miyoshi, D. Yoshidome, K. Fukase, *Bioorg. Med. Chem.* **2017**, *25*, 2844–2850; m) Y. Manabe, K. Hizume, Y. Takakura, S. Takamatsu, E. Miyoshi, Y. Kamada, R. Hurtado-Guerrero, K. Fukase, *Synlett* **2024**, *35*, 1273–1278.

[17] a) C. D. Rillahan, A. Antonopoulos, C. T. Lefort, R. Sonon, P. Azadi, K. Ley, A. Dell, S. M. Haslam, J. C. Paulson, *Nat. Chem. Biol.* **2012**, *8*, 661–668; b) N. M. Okeley, S. C. Alley, M. E. Anderson, T. E. Boursalian, P. J. Burke, K. M. Emmerton, S. C. Jeffrey, K. Klussman, C.-L. Law, D. Sussman, B. E. Toki, L. Westendorf, W. Zeng, X. Zhang, D. R. Benjamin, P. D. Senter, *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 5404–5409; c) Y. Kizuka, M. Nakano, Y. Yamaguchi, K. Nakajima, R. Oka, K. Sato, C.-T. Ren, T.-L. Hsu, C.-H. Wong, N. Taniguchi, *Cell Chem. Biol.* **2017**, *24*, 1467–1478.e5.

[18] a) M. Wang, Z. Zhang, M. Chen, Y. Lv, S. Tian, F. Meng, Y. Zhang, X. Guo, Y. Chen, M. Yang, J. Li, T. Qiu, F. Xu, Z. Li, Q. Zhang, J. Yang, J. Sun, H. Zhang, H. Zhang, H. Li, W. Wang, *Cell Death Dis.* **2023**, *14*, 495; b) Y. Lv, Z. Zhang, M. Wang, Y. Wang, M. Chen, J. Jia, Y. Guo, K. Wang, Z. Li, W. Wang, H. Li, *Bioorg. Chem.* **2024**, *149*, 107492.

[19] a) M. C. Bryan, L. V. Lee, C.-H. Wong, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3185–3188; b) C. D. Rillahan, S. J. Brown, A. C. Register, H. Rosen, J. C. Paulson, *Angew. Chem. Int. Ed.* **2011**, *50*, 12534–12537.

[20] a) L. R. McLean, Y. Zhang, H. Li, Z. Li, U. Lukaszczyk, Y.-M. Choi, Z. Han, J. Prisco, J. Fordham, J. T. Tsay, S. Reiling, R. J. Vaz, Y. Li, *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6717–6720; b) S. Madonna, C. Béclin, Y. Laras, V. Moret, A. Marcowycz, D. Lamoral-Theys, J. Dubois, M. Barthelemy-Requin, G. Lenglet, S. Depauw, T. Cresteil, G. Aubert, V. Monnier, R. Kiss, M.-H. David-Cordonnier, J.-L. Kraus, *Eur. J. Med. Chem.* **2010**, *45*, 623–638; c) Z. V. Boskovic, M. M. Kemp, A. M. Freedy, V. S. Viswanathan, M. S. Pop, J. H. Fuller, N. M. Martinez, S. O. Figueroa Lazú, J. A. Hong, T. A. Lewis, D. Calarese, J. D. Love, A. Vetere, S. C. Almo, S. L. Schreiber, A. N. Koehler, *ACS Chem. Biol.* **2016**, *11*, 1844–1851.

[21] a) A. García-García, L. Ceballos-Laita, S. Serna, R. Artschwager, N. C. Reichardt, F. Corzana, R. Hurtado-Guerrero, *Nat. Commun.* **2020**, *11*, 973; b) M. A. Järvå, M. Dramicanin, J. P. Lingford, R. Mao, A. John, K. E. Jarman, R. Grinter, E. D. Goddard-Borger, *J. Biol. Chem.* **2020**, *295*, 6677–6688.

[22] N. Shindo, H. Fuchida, M. Sato, K. Watari, T. Shibata, K. Kuwata, C. Miura, K. Okamoto, Y. Hatsuyama, K. Tokunaga, S. Sakamoto, S. Morimoto, Y. Abe, M. Shiroishi, J. M. M. Caaveiro, T. Ueda, T. Tamura, N. Matsunaga, T. Nakao, S. Koyanagi, S. Ohdo, Y. Yamaguchi, I. Hamachi, M. Ono, A. Ojida, *Nat. Chem. Biol.* **2019**, *15*, 250–258.

[23] C. D. Rillahan, A. Antonopoulos, C. T. Lefort, R. Sonon, P. Azadi, K. Ley, A. Dell, S. M. Haslam, J. C. Paulson, *Nat. Chem. Biol.* **2012**, *8*, 661–668.

[24] F. Ciardiello, G. Tortora, *New Engl. J. Med.* **2008**, *358*, 1160–1174.

[25] A. R. Timerbaev, C. G. Hartinger, S. S. Aleksenko, B. K. Keppler, *Chem. Rev.* **2006**, *106*, 2224–2248.

[26] L. Guandalini, M. V. Martino, L. Di Cesare Mannelli, G. Bartolucci, F. Melani, R. Malik, S. Dei, E. Floriddia, D. Manetti, F. Orlandi, E. Teodori, C. Ghelardini, M. N. Romanelli, *Bioorg. Med. Chem. Lett.* **2015**, *25*, 1700–1704.