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Discovery of Highly Potent Human Deoxyuridine Triphosphatase Inhibitors Based on the Conformation Restriction Strategy

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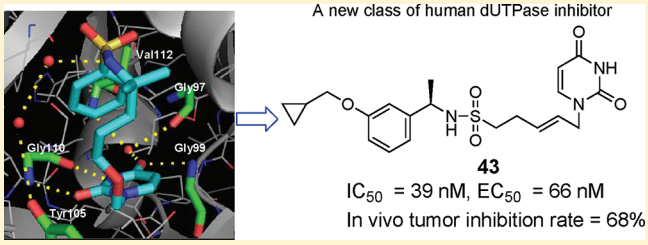
Supporting Information

ABSTRACT: Human deoxyuridine triphosphatase (dUTPase) inhibition is a promising approach to enhance the efficacy of thymidylate synthase (TS) inhibitor based chemotherapy. In this study, we describe the discovery of a novel class of human dUTPase inhibitors based on the conformation restriction strategy. On the basis of the X-ray cocrystal structure of dUTPase and its inhibitor compound **7**, we designed and synthesized two conformation restricted analogues, i.e., compounds **8** and **9**. These compounds exhibited increased in vitro potency compared with the parent compound **7**. Further structure−activity relationship (SAR) studies identified a compound **43** with the highest in vitro potency (IC_{50} = 39 nM, EC_{50} = 66 nM). Furthermore, compound **43** had a favorable oral PK profile and exhibited potent antitumor activity in combination with 5-fluorouracil (5-FU) in the MX-1 breast cancer xenograft model. These results suggested that a dUTPase inhibitor may have potential for clinical usage.



INTRODUCTION

Thymidylate synthase (TS) inhibitors such as 5-fluorouracil (5-FU), its analogues, and folate analogues are widely used in clinical chemotherapy.[1−4] However, acquired and intrinsic resistance still remains a limitation to the clinical use of TS inhibitors.[5] Therefore, novel therapeutic strategies to improve the efficacy of TS inhibitor based chemotherapy are urgently required.

TS inhibitors lead to rapid depletion of the cellular 2′-deoxyuridine 5′-triphosphate (dTTP) pool, inducing thymineless death. In addition, TS inhibition results in the accumulation of 2′-deoxyuridine 5′-monophosphate (dUMP), which may subsequently lead to increased levels of 2′-deoxyuridine 5′-triphosphate (dUTP).[6,7] dUTP can be misincorporated into DNA in place of dTTP during replication and repair by DNA polymerase.[8] High cellular dUTP/dTTP ratios induce futile cycles of misincorporation, which eventually lead to DNA strand breaks and cell death. This is one of the key antitumor mechanisms of TS inhibitors.[9−13]

dUTPase catalyzes the hydrolysis of dUTP to dUMP and has two functions in nucleotide metabolism: it decreases the number of intracellular dUTP pools to prevent misincorporation of uracil instead of thymine into DNA, and it supplies the substrate dUMP for TS, which is responsible for an important de novo nucleotide metabolism pathway in DNA synthesis.[14−17]

Since dUTPase can also hydrolyze FdUTP,[18] increased dUTPase activity induces resistance to 5-FU and its analogues. Genetically induced expression of dUTPase confers resistance to 5-fluoro-2′-deoxyuridine (FdUrd) in human tumor cells,[19−21] and small interfering RNA (siRNA) silencing of dUTPase in SW620 and MCF-7 cells significantly enhances the growth inhibition activity of FdUrd by perturbing (F)dUTP/dTTP levels.[12] On the other hand, a high level of dUTPase expression is associated with resistance to not only 5-FU and its analogues but folate analogues.[22]

Several clinical studies show that nuclear dUTPase levels correlate with poor survival outcomes in colorectal cancer[23] and hepatocelluar carcinoma (HCC).[24] These findings demonstrate that human dUTPase inhibitors may be useful as clinical medicines that enhance anticancer activity of TS inhibitors. Thus, efforts to obtain efficient dUTPase inhibitors have resulted in the development of several compounds (Figure 1).[25−34] These inhibitors had low K_i value against *Escherichia coli* or *Plasmodium faliciparum* dUTPase and were effectively used for solving the X-ray structure of the enzyme. However, these inhibitors appear to be ineffective for chemotherapeutic usage owing to high polarity or insufficient human dUTPase inhibition activity.

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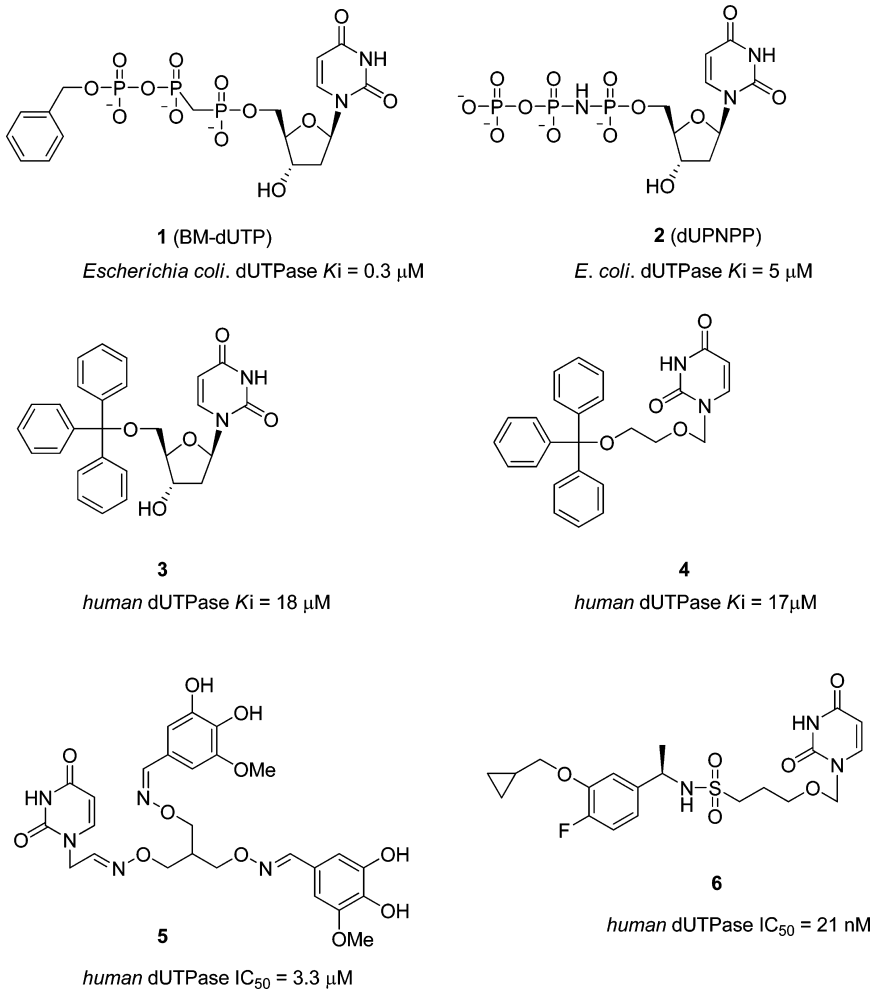
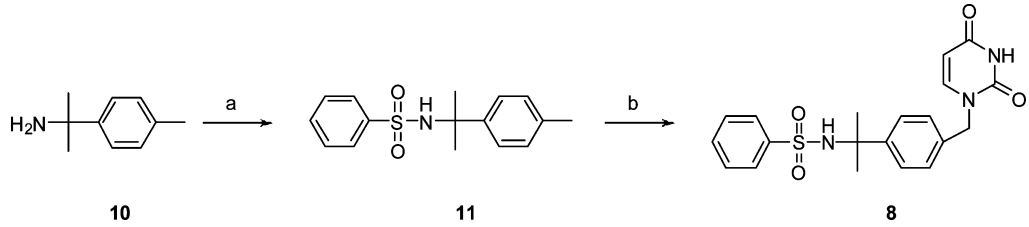


Figure 1. Structure of previously reported human dUTPase inhibitors.

Scheme 1. Synthesis of Compound 8[a]



[a]Reagents and conditions: (a) $PhSO_2Cl$, Et_3N, CH_2Cl_2, room temp, 2 h; (b) (1) NBS, AIBN, CCl_4, reflux, 0.5 h, (2) $(TMS)_2$uracil, I_2, DCE, reflux, 3 h.

We previously reported the development of highly potent dUTPase inhibitors.[35−37] Among them, compound **6** not only significantly enhances the growth inhibition activity of FdUrd against HeLa S3 cells in vitro but also shows robust antitumor activity in combination with 5-FU in vivo.

In this paper, we have described a new class of dUTPase inhibitors. We designed and synthesized conformation restricted dUTPase inhibitors based on the reported X-ray cocrystal structures (PDB code 3ARN[36]) and evaluated the inhibition activity against human dUTPase and in vivo antitumor activity in combination with 5-FU.

CHEMISTRY

The target compounds in this study were synthesized as depicted in Schemes 1−6. Compound **8** was synthesized by the method shown in Scheme 1. Briefly, the commercially available amine **10** was treated with benzenesulfonyl chloride to give compound **11**. After treatment of compound **11** with *N*-bromosuccinimide (NBS) in the presence of 2,2′-azobisisobutyronitrile (AIBN), the obtained benzyl bromide was coupled with $(TMS)_2$uracil to give **8**.

The synthesis of compound **9** is shown in Scheme 2. Moffat oxidation of commercially available compound **12** and subsequent Horner−Emmons olefination afforded compound **13**. After reduction of the olefin moiety of compound **13** using palladium on carbon under hydrogen atmosphere, treatment of

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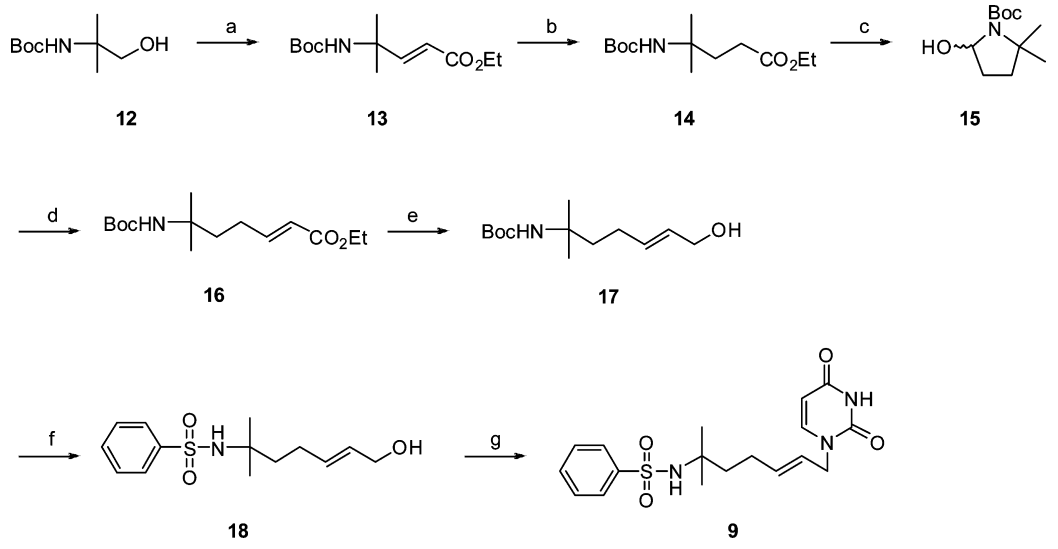
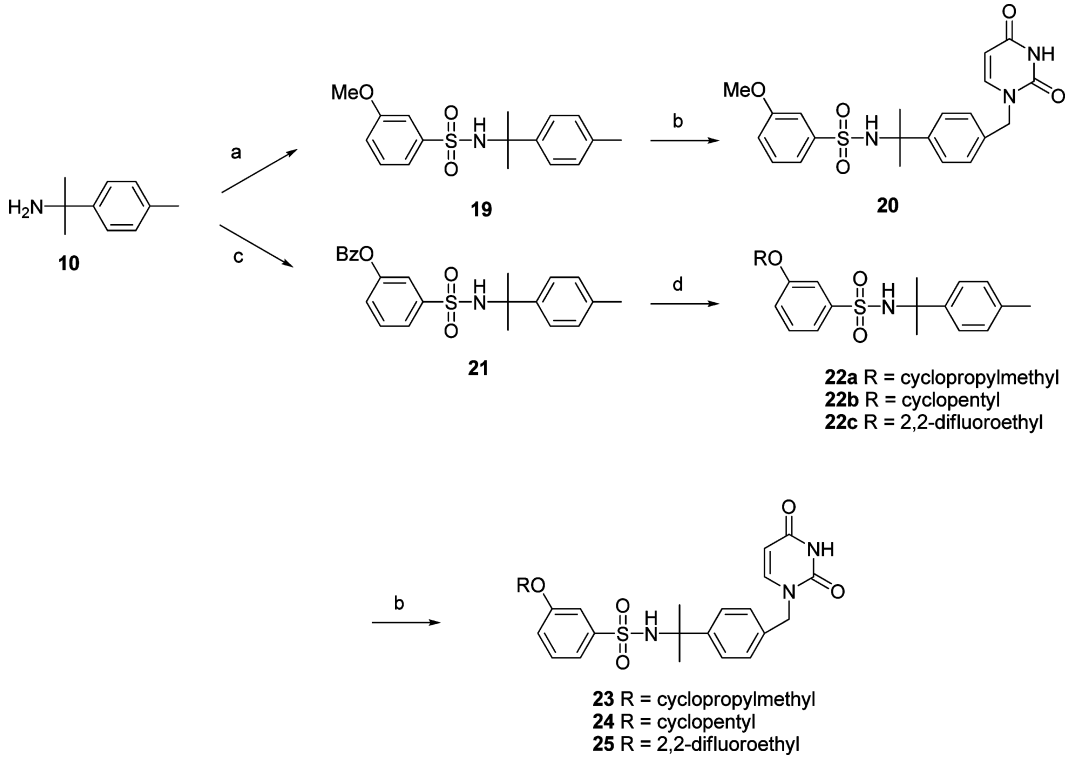
Scheme 2. Synthesis of Compound 9[a]



[a]Reagents and conditions: (a) (1) TFA, pyridine, EDC, DMSO, toluene, room temp, 30 min, (2) diethyl phosphonoacetate, NaH, THF, 75 °C, 1 h; (b) H_2, Pd/C, AcOEt, room temp, 4 h; (c) 2.0 M $LiBH_4$ in THF, THF, room temp, 16 h; (d) ethyl (triphenylphosphoranylidene)acetate, toluene, reflux, 18 h; (e) 1.0 M DIBAL-H in THF, THF, −78 °C, 2 h ; (f) (1) 4 N HCl/dioxane, room temp, 50 min, (2) $PhSO_2Cl$, Et_3N, MgO, THF, H_2O, room temp, 2 h; (g) (1) CBr_4, PPh_3, THF, room temp,1 h, (2) $(TMS)_2$uracil, I_2, DCE, reflux, 3 h.

Scheme 3. Synthesis of Compound 20 and Its Derivatives 23−25[a]



[a]Reagents and conditions: (a) 3-MeOPhSO_2Cl, Et_3N, CH_2Cl_2, room temp, 2 h; (b) (1) NBS, AIBN, CCl_4, reflux, 0.5 h, (2) $(TMS)_2$uracil, I_2, DCE, reflux, 3 h; (c) 3-BzOPhSO_2Cl, Et_3N, CH_2Cl_2, room temp, 2 h; (d) (1) 40% $MeNH_2$ in MeOH, room temp, 20 min, (2) RX, K_2CO_3, DMF, 90 °C, 16 h.

the resulting compound **14** with lithium borohydride ($LiBH_4$) afforded compound **15**. The Wittig reaction of compound **15** with ethyl (triphenylphosphoranylidene)acetate, followed by reduction of the ester group of the resulting compound **16**, afforded alcohol **17**. Removal of the Boc group from compound **17** and treatment of the resulting amine with benzenesulfonyl chloride afforded compound **18**. After bromination of compound **18** with carbon tetrabromide and triphenylphosphine, coupling of the resulting brominated compound with $(TMS)_2$uracil gave compound **9**.

Compound **20** and its derivatives **23−25** were synthesized in two or three steps, as shown in Scheme 3. These compounds

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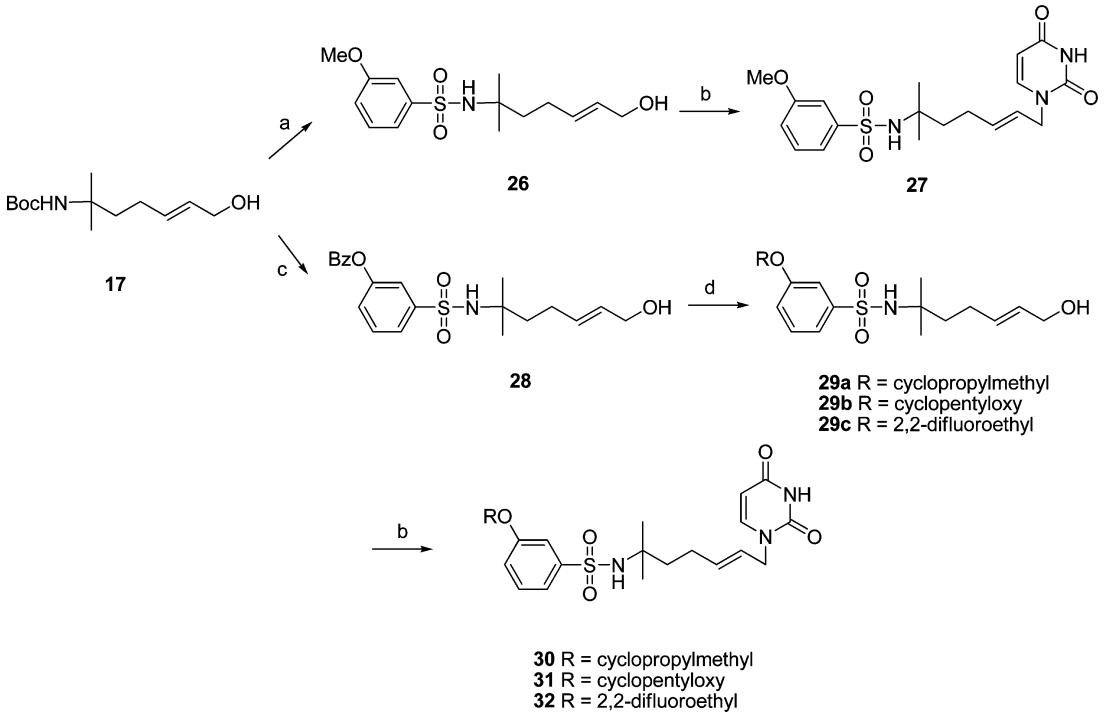
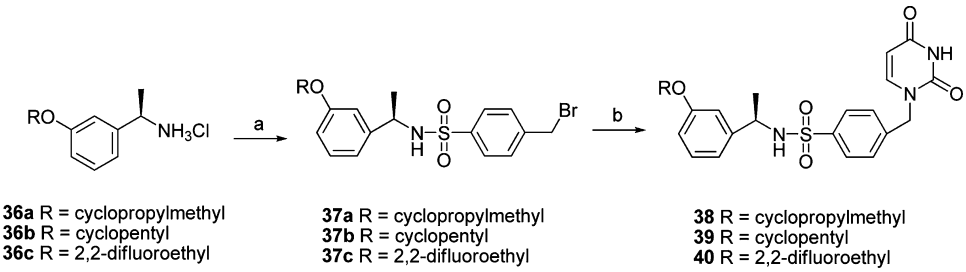
Scheme 4. Synthesis of Compound 27 and Its Derivatives 30−32[a]



[a]Reagents and conditions: (a) (1) 4 N HCl/dioxane, room temp, 50 min, (2) 3-MeOPhSO$_2$Cl, Et$_3$N, MgO, THF, H$_2$O, room temp, 2 h; (b) (1) CBr$_4$, PPh$_3$, THF, room temp, 1 h, (2) (TMS)$_2$uracil, I$_2$, DCE, reflux, 3 h; (c) (1) 4 N HCI/dioxane, room temp, 50 min, (2) 3-BzOPhSO$_2$Cl, Et$_3$N, MgO, THF, H$_2$O, room temp, 2 h; (d) (1) 40% MeNH$_2$ in MeOH, room temp, 20 min, (2) RX, K$_2$CO$_3$, DMF, 90 °C, 16 h.

Scheme 5. Synthesis of Compounds 38−40[a]



[a]Reagents and conditions: (a) 4-(bromomethyl)benzenesulfonyl chloride, Et$_3$N, CH$_2$Cl$_2$, 0 °C, 1 h; (b) (TMS)$_2$uracil, I$_2$, DCE, reflux, 5 h.

were synthesized in a manner similar to that shown in Scheme 1.

Compound **27** and its derivatives **30−32** were synthesized from intermediate **17**, as shown in Scheme 4. These compounds were synthesized in a manner similar to that shown in Scheme 2.

The synthesis of compounds **38−40** is shown in Scheme 5. Procedures for the synthesis of chiral amines **36b−c** are detailed in the Supporting Information. Briefly, chiral amine **36a** was treated with 4-(bromomethyl)benzenesulfonyl chloride, and the obtained benzyl bromide was coupling with (TMS)$_2$uracil to give compounds **38−40**. Compounds **39** and **40** were synthesized in a similar manner.

In Scheme 6, synthesis of compounds **43−45** is detailed. Procedures for the synthesis of intermediates **41a−c** are detailed in the Supporting Information. The Mitunobu reaction of **41a** with *N*-3-benzoyluracil provided compound **42a**. Deprotection of the benzoyl and MOM group of **42a** led to the production of compound **43**. Compounds **44** and **45** were synthesized in a similar manner.

RESULTS AND DISCUSSION

Design of the Conformation Restricted Analogues of Compound 7. The conformational restriction of a flexible ligand has often been a promising strategy to increase the potency for a given target protein in drug development because the entropic loss associated with the ligand adopting a preferred binding conformation can be minimized.[38−41] We previously reported the X-ray cocrystal structure of human dUTPase with compound **7** (PDB code 3ARN[36]) that has potent inhibition activity against human dUTPase (IC_{50} = 3.9 μM) (Figure 2). From this X-ray cocrystal structure, we confirmed that compound **7** adopts a folded conformation and is stacked between the uracil ring and its terminal phenyl ring. The central flexible linker moiety, i.e., -O-CH$_2$-CH$_2$-CH$_2$-C(Me)$_2$-, aided formation of the bent structure. We previously noted that the terminal phenyl ring of **7** occupies the same region as the

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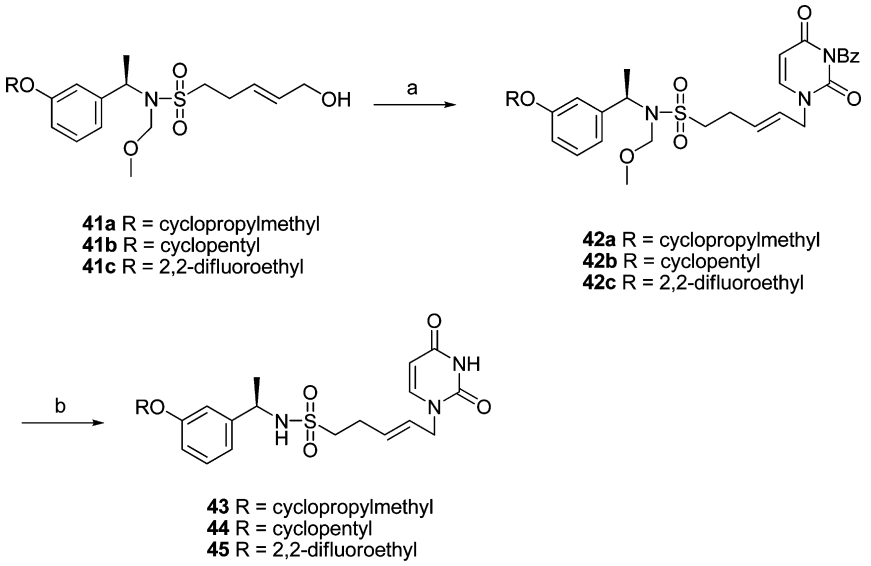
Scheme 6. Synthesis of Compounds 43−45[a]



[a]Reagents and conditions:(a) *N*-3-benzoyluracil, PPh_3, DEAD, THF, room temp, 2 h; (b) (1) 40% $MeNH_2$ in MeOH, room temp, 1 h, (2) 4 N HCl/dioxane, dioxane, room temp, 4 h.

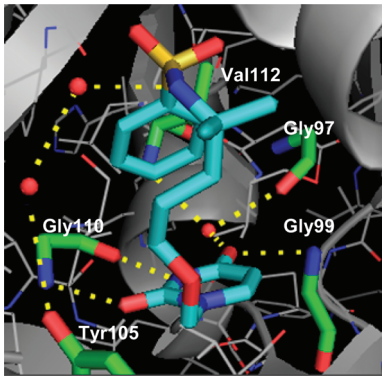


Figure 2. X-ray cocrystal structure of compound **7** (cyan) with human dUTPase (PDB code 3ARN[36]).

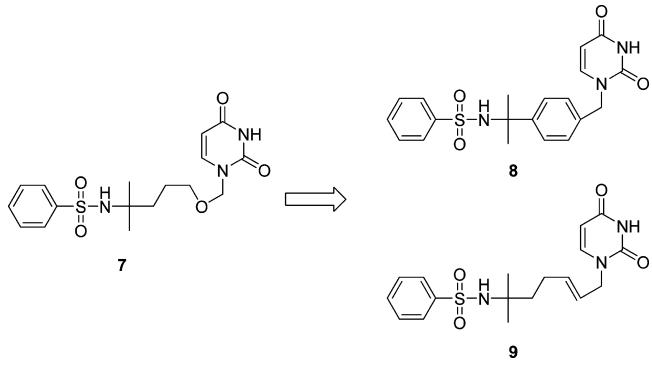


Figure 3. Design of the conformation restricted analogues as human dUTPase inhibitors.

Phe158 residue of the enzyme.[35] Mol and co-workers reported that capping each of the enzyme's active sites by the flexible C-terminal tail is critical for human dUTPase activity.[42] On the basis of this structural information, we proposed that this flexible linker moiety can be conformationally restricted into the bioactive conformation by replacing it with a *p*-phenylene ring or a *trans*-alkenylene group, in which the positions of the uracil and the terminal phenyl ring should be similar to those in compound **7**. On the basis of this hypothesis, we designed conformation restricted analogues **8** and **9** that have a rigid *p*-phenylene ring or a *trans*-alkenylene group (Figure 3).

We performed a molecular modeling study of conformation restricted analogues **8** and **9** based on the crystallographic pose of compound **7** (Figure 4A and Figure 4B). In modeling analysis, the aromatic stacking interaction between the uracil moiety and the terminal phenyl rings was conserved (Figure 4C).

We assayed the enzyme inhibition activity of compounds **8** and **9** and their in vitro growth inhibition in combination with FdUrd against Hela S3 cells. Conformation restricted analogues **8** and **9** demonstrated an almost 2-fold increment in enzyme inhibition activity and enhanced growth inhibition activity of FdUrd against Hela S3 cells compared with the parent compound **7** as expected (Table 1).

Further Improvement of Compounds 8 and 9. Next, to identify further potent inhibitors, we investigated the substitution effect at the terminal phenyl ring of compounds **8** and **9**. In our previous work describing the structure−activity relationship (SAR) of compound **7**, we discovered that introduction of a hydrophobic alkoxy group at the meta-position of the terminal phenyl ring of **7** is highly conducive to human dUTPase inhibitory activity[36] (approximately 2 orders). Accordingly, using the same strategy, we designed and synthesized compounds **20**, **23**−**25**, **27**, and **30**−**32** having an alkoxy group at the meta-position of the terminal phenyl ring.

Introduction of bulky lipophilic groups such as cyclopropylmethoxy, cyclopentyloxy, and 2,2-difluoroethoxy group into the meta-position of the terminal phenyl ring dramatically increased both enzyme inhibition activity and enhanced the growth inhibition activity of FdUrd against Hela S3 cells as expected (Table 2). It is suggested that the meta-subsutituent on their terminal phenyl ring contributes important hydrophobic interaction with the enzyme.

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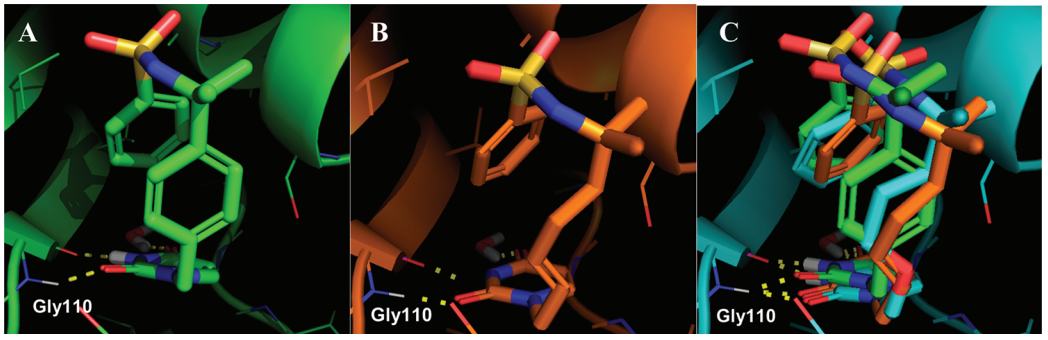


Figure 4. (A) Proposed model for compound **8** (green) binding to the active site pocket. (B) Proposed model for compound **9** (orange) binding to the active site pocket. (C) Overlay of compounds **7** (cyan), **8**, and **9** complexed to human dUTPase.

Table 1. Bilological Activity of Compounds 7, 8, and 9

compd	IC_{50} (μM)[a,c]	EC_{50} (μM)[b,c]
7	3.90 ± 0.63	5.10 ± 0.31
8	1.80 ± 0.20	2.60 ± 0.07
9	1.60 ± 0.05	2.60 ± 0.36

[a]Concentration of each compound required to inhibit 50% of the amount of [5-^{3}H]dUMP produced by human dUTPase. [b]Concentration of each compound that reduces the T/C (%) of FdUrd (1 μM) against HeLa S3 to half in 24 h. [c]IC_{50} and EC_{50} data represent the average of three independent assays, and errors are given as standard deviations.

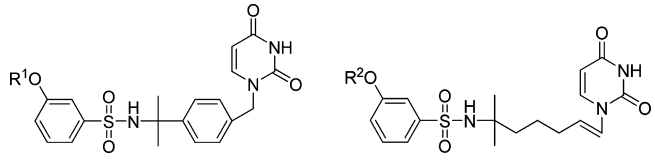
Although these compounds have favorable biological activity, none of these could be solidified, making them difficult for further development. As described in our previous paper, the conversion of the linker part of compound **33** to an alternative linker resolved this physicochemical issue without disrupting enzyme inhibition activity. Furthermore, introduction of an (*R*)-monomethyl group at the benzyl position of compound **34** significantly contributed to its increased enzyme inhibition activity (Figure 5). This result suggested that the substituent of the (*R*)-tail is more likely to maintain close contact with the enzyme.

On the basis of the SAR in our previous study, we designed and synthesized compounds **38**–**40** and **43**–**45** with (*R*)-methylated benzyl groups, as shown in Schemes 5 and 6, respectively. Unexpectedly, compounds **38**–**40** were approximately 4-fold to 13-fold less active than compounds **23**–**25** (Table 3). The terminal phenyl ring of the reverse sulfonamide compounds **38**–**40**, which is essential for efficient dUTPase inhibition activity, may not have been located at a suitable position in the active site of the enzyme because of the rigidity of compounds. On the other hand, compounds **43**–**45** retained enzyme inhibition activity compared with parent compounds **30**–**32** (Table 4). These compounds had favorable physicochemical properties (both compounds were solidified). Among them, compound **43** has the greatest in vitro potency (IC_{50} = 39 nM, EC_{50} = 66 nM). On the other hand, compound **43** alone had little effect on cell growth (IC_{50} = 38.2 μM). Therefore, we selected compound **43** for further study.

In Vivo Pharmacokinetics (PK) Profile of Compound 43. Compound **43** was evaluated for preliminary pharmacokinetics profile in mice. These results are summarized in Table 5. Compound **43** was well absorbed into mice plasma after oral administration. These PK data showed that compound **43** has a desirable profile for in vivo study.

Antitumor Activity of Compound 43 against the MX-1 Breast Cancer Xenograft Model in Mice. We next evaluated the antitumor activity of compound **43** in combination with 5-FU in vivo. Compound **43** was administered orally with a continuous infusion of 5-FU into the MX-1 xenograft model in mice. Compound **43** dramatically increased the efficacy of 5-FU (inhibition rate IR = 68%)

Table 2. Bilological Activity for Meta-Substituted Analogues of Compounds 8 and 9



compd	R^1	R^2	IC_{50} (μM)[a,c]	EC_{50} (μM)[b,c]
20	CH_3		1.27 ± 0.07	3.13 ± 0.44
23	cyclopropylmethyl		0.073 ± 0.012	0.14 ± 0.02
24	cyclopentyl		0.031 ± 0.002	0.12 ± 0.005
25	2,2-difluoroethyl		0.073 ± 0.009	0.10 ± 0.03
27		CH_3	0.34 ± 0.02	0.45 ± 0.04
30		cyclopropylmethyl	0.028 ± 0.004	0.071 ± 0.005
31		cyclopentyl	0.033 ± 0.003	0.20 ± 0.005
32		2,2-difluoroethyl	0.026 ± 0.001	0.049 ± 0.002

[a]Concentration of each compound required to inhibit 50% of the amount of [5-^{3}H]dUMP produced by human dUTPase. [b]Concentration of each compound that reduces the T/C (%) of FdUrd (1 μM) against HeLa S3 to half in 24 h. [c]IC_{50} and EC_{50} data represent the average of three independent assays, and errors are given as standard deviations.

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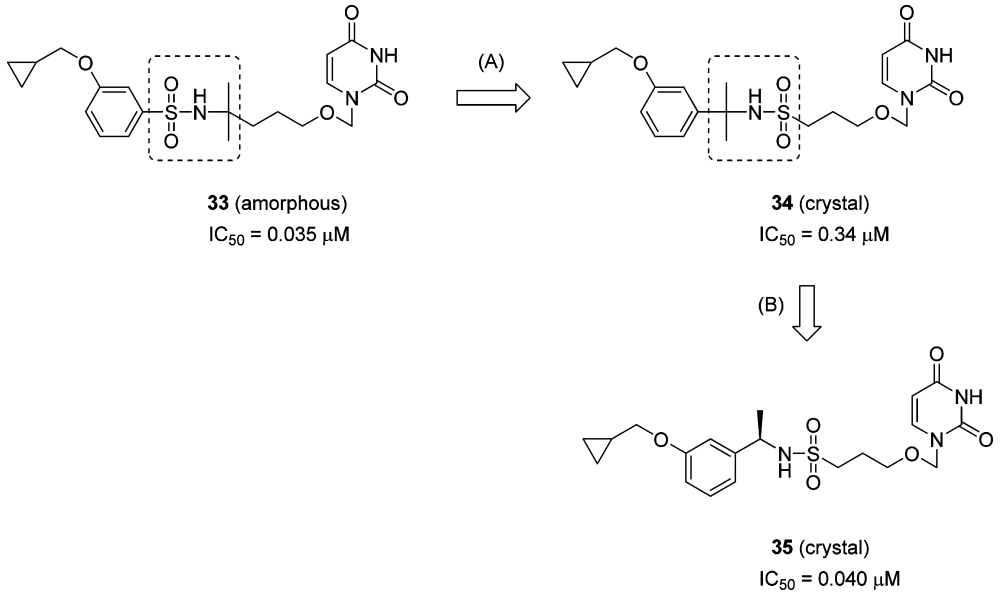
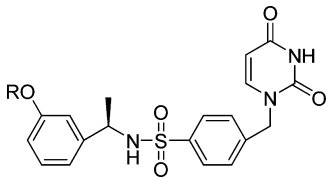


Figure 5. (A) Replacement of the linker moiety conferred favorable physicochemical properties in our chemical series. (B) (*R*)-Monomethylated compound showed increased inhibition enzyme activity.

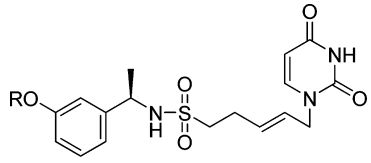
Table 3. Biological Data of Compounds 38−40



compd	R	IC_{50} (μM)[a,c]	EC_{50} (μM)[b,c]
38	cyclopropylmethyl	0.82 ± 0.02	0.93 ± 0.09
39	cyclopentyl	0.43 ± 0.06	0.61 ± 0.02
40	2,2-difluoroethyl	0.41 ± 0.05	0.61 ± 0.03

[a]Concentration of each compound required to inhibit 50% of the amount of [5-^{3}H]dUMP produced by human dUTPase. [b]Concentration of each compound that reduces the *T*/*C* (%) of FdUrd (1 μM) against HeLa S3 to half in 24 h. [c]IC_{50} and EC_{50} data represent the average of three independent assays, and errors are given as standard deviations.

Table 4. Biological Data of Componds 43−45



compd	R	IC_{50} (μM)[a,c]	EC_{50} (μM)[b,c]
43	cyclopropylmethyl	0.039 ± 0.003	0.066 ± 0.002
44	cyclopentyl	0.041 ± 0.003	0.15 ± 0.01
45	2,2-difluoroethyl	0.082 ± 0.007	0.096 ± 0.009

[a]Concentration of each compound required to inhibit 50% of the amount of [5-^{3}H]dUMP produced by human dUTPase. [b]Concentration of each compound that reduces the *T*/*C* (%) of FdUrd (1 μM) against HeLa S3 to half in 24 h. [c]IC_{50} and EC_{50} data represent the average of three independent assays, and errors are given as standard deviations.

without causing significant weight loss or toxicity compared with the vehicle (2.5% DMA, 2.5% Tween 80, 10% Cremophor, and 0.5% HPMC) treated group (Table 6, Figure 6A, Figure 6B). On the other hand, compound **43** exhibited no antitumor activity when administered alone (Table 6). These results indicate that this conformation restricted dUTPase inhibitor may provide high efficacy for treating cancer patients when used in combination with TS inhibitor.

Table 5. Pharmacokinetic Profile of 43 after Oral Administration (po)[a]

compd	AUC_{0-t} (μM·h)	C_{max} (μM)	$T_{1/2}$ (h)	T_{max} (h)
43	19.5	19.5	0.89	0.5

[a]Balb/cA male mice (n = 2) was dosed at 50 mg/kg. The po formulation contained 2.5% DMA, 2.5% Tween 80, and 10% Cremophor EL.

CONCLUSION

We have described the discovery of conformation restricted human dUTPase inhibitors. On the basis of the X-ray cocrystal structure of lead compound **7** and human dUTPase, we designed and synthesized compounds **8** and **9** having rigid linkers such as a *p*-phenylene ring or a *trans*-alkenylene group instead of the flexible linker. Modeling study of these compounds suggested that the terminal phenyl ring can be located at the same region as that of compound **7**. In biological assay, these compounds show more potent activity than the parent compound **7**, as expected. Further elaboration of the SAR around compounds **8** and **9** gave a highly potent human dUTPase inhibitor **43**. Compound **43** is one of the most potent human dUTPase inhibitors known so far, and it shows excellent antitumor activity in combination with 5-FU in the MX-1 xenograft model. Development of this inhibitor may validate dUTPase inhibitors as a potential new regiment in the combination chemotherapy with TS inhibitors for treating human cancer.

EXPERIMENTAL SECTION

Chemistry. All commercially available reagents and solvents were used without further purification unless otherwise specified. All

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Table 6. Antitumor Activity of Compound 43 in Combination with 5-FU in the MX-1 Xenograft Model

drug	dose (mg kg^{-1} day^{-1})	treatment	TV,[a] mean ± SD (mm^3)	RTV,[b] mean ± SD	IR[c] (%)
control			2048 ± 693.7	11.4 ± 3.74	
5-FU	15	CI	1645 ± 322.6	9.13 ± 1.35	20
43	300	po	2819 ± 776.3	14.7 ± 2.54	−29
5-FU/**43**	15/300	CI/po	746 ± 301.1	3.66 ± 1.30**,##	68

[a]Tumor volume (TV) on day 15 was calculated according to the following formula: TV (mm^3) = (width)^2(length)/2. [b]Relative tumor volume (RTV) on day 15 was calculated as the ratio of TV on day 15 to that on day 0 according to the following formula: RTV = (TV on day 15)/(TV on day 0). **: $p < 0.01$, Dunnett's test compared with the control group. ##: $p < 0.01$, Student's t-test compared with the 5-FU group. [c]Inhibition rate (IR) of tumor growth on day 15 on the basis of RTV was calculated according to the following formula: IR (%) = [1 − (mean RTV of the treated group)/(mean RTV of the control group)] × 100.

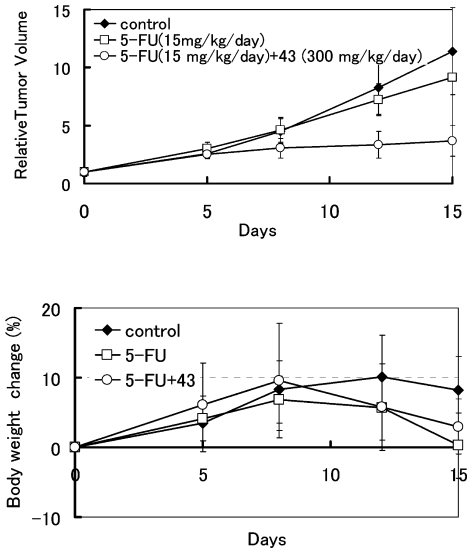

Figure 6. (A, top) Antitumor activity of **43** in combination with 5-FU in the MX-1 xenograft model. Relative tumor volume (RTV) is expressed as the mean ± SD of at least three independent experiments. (B, bottom) Body weight change (%) is expressed as the mean ± SD.

reactions were performed under an inert nitrogen atmosphere unless otherwise specified. ^{1}H NMR spectra were recorded on a JEOL JNM-EX-270 (270 MHz) or JEOL JNM-LA-400 (400 MHz) spectrometer, and ^{13}C NMR spectra were recorded on a JEOL JNM-LA-400 (100 MHz) spectrometer. Chemical shifts are given in parts per million (ppm, δ) with tetramethylsilane as the internal standard, and coupling constants (J) are given in hertz (Hz). Splitting patterns and apparent multiplicities are designated as s, singlet; d, doublet; dd, double doublet; t, triplet; dt, doublet triplet; q, quartet; quin, quintet; m, multiplet; brs, broad singlet. Analytical thin layer chromatography (TLC) was performed on silica gel 60 F_{254} precoated plates (Merck). Column chromatography was performed on Merck silica gel 60 (230–400 mesh). Optical rotation was determined using Horiba SEPA-200 polarimeter. High-resolution mass spectra were recorded either on a JEOL JMS-700 (FAB) or a on Waters micromass Q-Tof-2 (TOF) instrument. The purity of all final compounds was determined by combustion analysis or high pressure liquid chromatography (HPLC); purity of at least 95% was found. Elemental analyses were performed using a Thermo Electron Corporation Flash EA 1112 series. Analytical HPLC was performed on a Shimazu Prominence system using L-column 2 ODS column (4.6 mm × 150 mm, 3 μm) with an 8 min linear gradient from 10% to 80% acetonitrile/10 mM phosphate buffer (pH 6.5) and a flow rate of 1.3 mL/min with UV detection at 220 nm (method A) and using a Shim-pack XR-ODS column (3.0 mm × 50 mm, 2.2 μm) with an 8 min linear gradient from 10% to 80% acetonitrile/10 mM phosphate buffer (pH 6.5) and a flow rate of 0.8 mL/min with UV detection at 220 nm (method B). The retention time of compound peak in HPLC is denoted as t_R.

***N*-(2-*p*-Tolylpropan-2-yl)benzenesulfonamide (11).** To a stirred solution of **10** (280 mg, 1.88 mmol) in CH_2Cl_2 (3.5 mL) were added Et_3N (250 μL, 1.79 mmol) and benzenesulfonyl chloride (205 μL, 1.60 mmol) at 0 °C, and the resulting mixture was stirred at room temperature for 2 h. The mixture was poured into H_2O and extracted with EtOAc two times. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel, eluting with hexane/EtOAc = 4/1 to afford the title compound (121 mg, 0.42 mmol, 22%) as a colorless oil. ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.62 (6H, s), 2.34 (3H, s), 7.03–7.19 (8H, m), 7.31–7.34 (1H, m).

***N*-(2-(4-((2,4-Dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)methyl)-phenyl)propan-2-yl)benzenesulfonamide (8).** A mixture of **11** (121 mg, 0.42 mmol), *N*-bromosuccinimide (78.0 mg, 0.44 mmol), and AIBN (2.5 mg, 0.015 mmol) in CCl_4 (3.0 mL) was heated to reflux at 90 °C for 0.5 h. The mixture was cooled to room temperature, poured into H_2O, and extracted with EtOAc two times. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford a crude brominated product.

To a suspension of 2,4-bis(trimethylsilyloxy)pyrimidine (150 mg, 0.58 mmol) in 1,2-dichloroethane (2.0 mL) was added a solution of the crude brominated product in 1,2-dichloroethane (1.0 mL) at room temperature. The resulting mixture was heated to reflux at 95 °C for 3 h. After cooling to room temperature, the mixture was poured into H_2O and extracted with EtOAc two times. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel, eluting with EtOAc to afford the title compound (39.1 mg, 0.098 mmol, 23% from **11**) as a colorless gum. ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.60 (6H, s), 4.84 (2H, s), 5.53 (1H, brs), 5.72 (1H, d, J = 7.9 Hz), 7.09–7.19 (3H, m), 7.27–7.38 (4H, m), 7.42–7.52 (1H, m), 7.65–7.69 (2H, m), 8.45 (1H, brs). ^{13}C NMR (100 MHz, DMSO-d_6) δ 29.7, 49.8, 57.2, 101.3, 125.8, 126.1, 126.8, 128.5, 131.4, 134.8, 143.2, 145.3, 145.7, 151.0, 163.7. Anal. Calcd for $C_{20}H_{21}N_3O_4S$: C, 60.13; H, 5.3; N, 10.52. Found: C, 60.00; H, 5.35; N, 10.33.

(*E*)-Ethyl 4-(*tert*-Butoxycarbonylamino)-4-methylpent-2-enoate (13). To a mixture of *tert*-butyl 1-hydroxy-2-methylpropan-2-ylcarbamate **12** (38.5 g, 203 mmol), trifluoroacetic acid (11.3 mL, 152 mmol), pyridine (24.6 mL, 305 mm) in DMSO (160 mL) and toluene (160 mL) was added EDC (116.8 g, 609 mmol) at room temperature. The resulting mixture was stirred at this temperature for 30 min and then poured into H_2O. The aqueous layer was extracted with EtOAc two times. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give a crude aldehyde.

To a stirred suspension of NaH (55% in oil, 10.7 g, 245 mmol) in THF (150 mL) was slowly added ethyl diethylphosphonoacetate (49.6 mL, 248 mmol) at 0 °C, and the resulting mixture was stirred at room temperature for 1 h. Then a solution of the crude aldehyde in THF (100 mL) was added to the mixture, and the whole was stirred at 75 °C for 1 h. The reaction mixture was quenched by the addition of saturated aqueous NH_4Cl, and the aqueous layer was extracted with EtOAc two times. The combined organic layer was washed with brine,

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dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel, eluting with hexane/EtOAc = 7/3 to afford the title compound (47.4 g, mmol, 91%, from **12**) as a colorless oil. ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.21−1.29 (3H, m), 1.41 (6H, s), 1.43 (9H, s), 4.11−4.23 (2H, m), 4.68 (1H, brs), 5.84 (1H, d, J = 15.8 Hz), 7.00 (1H, d, J = 15.8 Hz).

Ethyl 4-(*tert*-Butoxycarbonylamino)-4-methylpentanoate (14). A mixture of **13** (47.4 g, 184 mmol) and 10% palladium on activated carbon (3.0 g) in EtOAc (200 mL) was stirred under a hydrogen atmosphere (balloon) at room temperature for 4 h. The reaction mixture was filtered with a pad of Celite, and the pad was washed with EtOAc. The filtrate was concentrated under reduced pressure to give the title compound (40.0 g, 154 mmol, 84%) as a colorless oil. ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.24 (3H, t, J = 7.1 Hz), 1.26 (6H, s), 1.46 (9H, s), 1.96−2.05 (2H, m), 2.28−2.34 (2H, m), 4.12 (2H, q, J = 7.1 Hz), 4.43 (1H, brs). HRMS (FAB) calcd for $C_{13}H_{26}NO_4$ $[M + H]^+$ 260.1862, found 260.1857.

***tert*-Butyl 5-Hydroxy-2,2-dimethylpyrrolidine-1-carboxylate (15).** To a solution of **14** (17.4 g, 67.0 mmol) in THF (200 mL) was added $LiBH_4$ (2.0 M, 55.4 mL, 111 mmol) at room temperature. The reaction mixture was stirred at this temperature for 16 h. Then to the mixture was added water dropwise at the same temperature. The aqueous layer was extracted with EtOAc two times. The combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel, eluting with hexaneEtOAc = 3/7 to afford the title compound (5.90 g, 27.4 mmol, 41%) as a white solid. ^{1}H NMR (270 MHz, DMSO-d_6) δ 1.40 (6H, s), 1.47 (9H, s), 1.51−1.59 (2H, m), 1.96−2.05 (2H, m), 5.17−5.21 (1H, m). HRMS (FAB) calcd for $C_{11}H_{20}NO_3$ $[M - H]^-$ 214.1443, found 214.1440.

(*E*)-Ethyl 6-(*tert*-Butoxycarbonylamino)-6-methylhept-2-enoate (16). To a solution of **15** (940 mg, 4.37 mmol) in toluene (20 mL) was added ethyl (triphenylphosphoranylidene)acetate (1.74 g, 5.0 mmol), and the resultant was heated to reflux at 110 °C for 18 h. The mixture was concentrated under reduced pressure and the residue was purified by column chromatography on silica gel, eluting with hexane/EtOAc = 8/1 to afford the title compound (543 mg, 1.90 mmol, 43%) as a colorless oil. ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.25−1.31 (9H, m), 1.43 (9H, s), 1.78−1.85 (2H, m), 2.14−2.20 (2H, m), 3.38 (1H, brs), 4.17 (2H, q, J = 7.3 Hz), 5.79−5.86 (1H, m), 6.93−7.02 (1H, m). HRMS (FAB) calcd for $C_{15}H_{28}NO_4$ $[M + H]^+$ 286.2018, found 286.1998.

(*E*)-*tert*-Butyl 7-Hydroxy-2-methylhept-5-en-2-ylcarbamate (17). To a stirred solution of **16** (530 mg, 1.86 mmol) in THF (10 mL) was slowly added DIBAL-H in THF (1.0 M, 9.30 mL, 9.30 mmol) at −78 °C. The reaction mixture was stirred at −78 °C for 2 h. Brine and saturated aqueous Rochelle's salt were added to the mixture at −78 °C, and the resultant was stirred at room temperature for 16 h. The aqueous layer was extracted with EtOAc two times. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel, eluting with hexane/EtOAc = 3/1 to afford the title compound (452 mg, 1.86 mmol, quantitative) as a colorless oil. ^{1}H NMR (270 MHz, DMSO-d_6) δ 1.13 (6H, s), 1.36 (9H, s), 1.58−1.62 (2H, m), 1.87−1.93 (2H, m), 3.84−3.89 (2H, m), 4.53 (1H, t, J = 5.4 Hz), 5.41−5.59 (2H, m), 6.35 (1H, brs). HRMS (FAB) calcd for $C_{13}H_{26}NO_3$ $[M + H]^+$ 244.1913, found 244.1933.

(*E*)-*N*-(7-Hydroxy-2-methylhept-5-en-2-yl)-benzenesulfonamide (18). A solution of **17** (486 mg, 2.00 mmol) in 4 N HCl/dioxane (5.0 mL) was stirred at room temperature for 50 min. The mixture was concentrated under reduced pressure, and the residue was coevaporated with toluene four times and dissolved in THF (3.2 mL) and H_2O (800 μL). To this mixture were added Et_3N (585 μL, 4.20 mmol), MgO (400 mg, 9.93 mmol), and benzenesulfonyl chloride (293 μL, 2.30 mmol) at room temperature, and the resultant was stirred at the same temperature for 2 h. The mixture was filtered, and the filter cake was washed with EtOAc and H_2O. The filtrate was concentrated under reduced pressure, and the residue was partitioned between EtOAc and H_2O. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel, eluting with hexane/EtOAc = 1/1 to afford the title compound (128 mg, 0.57 mmol, 23% from **17**) as a colorless oil. ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.20 (6H, s), 1.59−1.64 (2H, m), 2.01−2.17 (2H, m), 4.05−4.09 (2H, m), 4.52 (1H, brs), 5.61−5.64 (2H, m), 7.50−7.55 (3H, m), 7.86−7.90 (2H, m). HRMS (FAB) calcd for $C_{14}H_{20}NO_3S$ $[M - H]^-$ 282.1164, found 282.1192.

(*E*)-*N*-(7-(2,4-Dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-2-methylhept-5-en-2-yl)benzenesulfonamide (9). To a solution of **18** (160 mg, 0.56 mmol) in THF (2.5 mL) were added PPh_3 (223 mg, 0.85 mmol) and CBr_4 (280 mg, 0.84 mmol) at room temperature, and the resulting mixture was stirred for 1 h. The mixture was concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel, eluting with hexane/EtOAc = 3/1) to give a crude brominated compound.

To a suspension of 2,4-bis(trimethylsilyloxy)pyrimidine (79.0 mg, 0.31 mmol) in 1,2-dichloroethane (1.0 mL) was added a solution of the crude brominated compound in 1,2-dichloroethane (1.0 mL) and iodine (catalyst) at room temperature. The resulting mixture was heated to reflux at 95 °C for 3 h. After cooling to room temperature, the mixture was poured into H_2O/saturated aqueous $Na_2S_2O_3$ and extracted with EtOAc two times. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel, eluting with EtOAc to afford the title compound (32.0 mg, 0.085 mmol, 15% from **18**) as a white foam. ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.17 (6H,s), 1.61−1.66 (2H, m), 2.06−2.17 (2H, m), 4.27 (2H, d, J = 6.2 Hz), 4.52 (1H, brs), 5.41−5.51 (1H, m), 5.64−5.71 (1H, m), 5.71 (1H, dd, J = 8.1, 2.7 Hz), 7.16 (1H, d, J = 8.1 Hz), 7.46−7.55 (3H, m), 7.86−7.91 (2H, m), 8.33 (1H, brs). ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.8, 27.7, 41.6, 49.6, 56.9, 102.3, 123.3, 126.9, 128.9, 132.2, 136.1, 143.4, 143.9, 150.8, 163.8. Anal. Calcd for $C_{18}H_{23}N_3O_4S{\cdot}0.3H_2O$: C, 56.47; H, 6.21; N, 10.98. Found: C, 56.76; H, 6.08; N, 11.04.

3-Methoxy-*N*-(2-*p*-tolylpropan-2-yl)benzenesulfonamide (19). To a stirred solution of **10** (350 mg, 2.35 mmol) in CH_2Cl_2 (3.5 mL) were added Et_3N (401 μL, 2.88 mmol) and 3-methoxybenzenesulfonyl chloride (398 μL, 2.53 mmol) at 0 °C, and the resulting mixture was stirred at room temperature for 2 h. The mixture was poured into H_2O and extracted with EtOAc two times. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel, eluting with hexane/EtOAc = 4/1 to afford the title compound (179 mg, 0.56 mmol, 24%) as a white solid. ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.64 (6H, s), 2.28 (3H, s), 3.76 (3H, s), 4.85 (1H, brs), 6.95−7.00 (3H, m), 7.04−7.06 (1H, m), 7.14−7.17 (2H, m), 7.26−7.29 (2H, m). HRMS (FAB) calcd for $C_{17}H_{20}NO_3S$ $[M - H]^-$ 318.1164, found 318.1153.

***N*-(2-(4-((2,4-Dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)methyl)-phenyl)propan-2-yl)-3-methoxybenzenesulfonamide (20).** **20** was prepared from **19** (174 mg, 0.54 mmol) as described for the preparation of **8**, colorless gum (21 mg, 0.053 mmol, 9%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.63 (6H, s), 3.77 (3H, s), 4.84 (2H, s), 5.24 (1H, brs), 5.72 (1H, d, J = 7.8 Hz), 6.94−7.34 (9H, m), 8.82 (1H, brs). ^{13}C NMR (100 MHz, CD_3OD) δ 30.5, 51.7, 56.0, 58.6, 102.6, 112.6, 119.1, 120.0, 127.5, 128.4, 130.7, 135.8, 145.3, 146.3, 147.1, 152.8, 160.8, 166.7. HRMS (TOF) calcd for $C_{21}H_{24}N_3O_5S$ $[M + H]^+$ 430.1437, found 430.1443. HPLC purity: 96.8%, t_R = 4.82 min (method B).

3-(*N*-(2-*p*-Tolylpropan-2-yl)sulfamoyl)phenyl Benzoate (21). To a stirred solution of **10** (1.49 g, 9.98 mmol) in CH_2Cl_2 (25 mL) were added Et_3N (1.46 mL, 10.5 mmol) and 3-(chlorosulfonyl)phenyl benzoate (2.08 g, 7.01 mmol) at 0 °C, and the resulting mixture was stirred at room temperature for 2 h. The mixture was poured into H_2O and extracted with EtOAc two times. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel, eluting with hexane/EtOAc = 4/1 to

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afford the title compound (2.07 g, 5.05 mmol, 51%) as a colorless gum. ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.67 (6H, s), 2.26 (3H, s), 4.88 (1H, brs), 6.98−7.01 (2H, m), 7.17−7.20 (2H, m), 7.33−7.39 (2H, m), 7.48−7.57 (4H, m), 7.64−7.71 (1H, m), 8.18−8.22 (2H, m). HRMS (FAB) calcd for $C_{23}H_{22}NO_4S$ $[M - H]^-$ 408.1270, found 408.1282.

3-(Cyclopropylmethoxy)-*N*-(2-*p*-tolylpropan-2-yl)-benzenesulfonamide (22a). A solution of **21** (287 mg, 0.70 mmol) in 40% $MeNH_2$ in MeOH (4.0 mL) was stirred at room temperature for 20 min. The mixture was concentrated under reduced pressure, and the residue was coevaporated with toluene two times and was then dissolved in DMF (4.0 mL). To the mixture were added K_2CO_3 (193 mg, 1.40 mmol) and (bromomethyl)cyclopropane (74.7 μL, 0.77 mmol) at room temperature. The resulting mixture was stirred at 90 °C for 16 h and then poured into H_2O. The aqueous layer was extracted with EtOAc two times. The combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel, eluting with hexane/EtOAc = 4/1 to afford the title compound (224 mg, 0.62 mmol, 89% from **21**) as a white solid. ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.32−0.38 (2H, m), 0.62−0.70 (2H, m), 1.20−1.29 (1H, m), 1.63 (6H, s), 2.28 (3H, s), 3.74 (2H, d, J = 7.0 Hz), 4.84 (1H, brs), 6.93−7.04 (4H, m), 7.11−7.14 (2H, m), 7.25−7.30 (2H, m). HRMS (FAB) calcd for $C_{20}H_{24}NO_3S$ $[M - H]^-$ 358.1477, found 358.1465.

3-(Cyclopentyloxy)-*N*-(2-*p*-tolylpropan-2-yl)-benzenesulfonamide (22b). **22b** was prepared from **21** (573 mg, 1.40 mmol) as described for the preparation of **22a**, white solid (519 mg, 1.39 mmol, 99%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.63 (6H, s), 1.64−1.69 (2H, m), 1.72−1.90 (6H, m), 2.28 (3H, s), 4.67−4.83 (1H, m), 4.80 (1H, brs), 6.93−7.00 (3H, m), 7.11−7.26 (5H, m). HRMS (FAB) calcd for $C_{21}H_{26}NO_3S$ $[M - H]^-$ 372.1633, found 372.1659.

3-(2,2-Difluoroethoxy)-*N*-(2-*p*-tolylpropan-2-yl)-benzenesulfonamide (22c). **22c** was prepared from **21** (573 mg, 1.40 mmol) as described for the preparation of **22a**, white solid (510 mg, 1.38 mmol, 99%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.65 (6H, s), 2.27 (3H, s), 4.08 (2H, td, J = 13.2, 4.3 Hz), 4.90 (1H, brs), 6.07 (1H, tt, J = 55.1, 4.1 Hz), 6.93−7.04 (4H, m), 7.11−7.14 (2H, m), 7.27−7.36 (2H, m). HRMS (FAB) calcd for $C_{18}H_{20}F_2NO_3S$ $[M - H]^-$ 368.1132, found 368.1161.

3-(Cyclopropylmethoxy)-*N*-(2-(4-((2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)methyl)phenyl)propan-2-yl)-benzenesulfonamide (23). **23** was prepared from **22a** (230 mg, 0.64 mmol) as described for the preparation of **8**, colorless gum (113 mg, 0.24 mmol, 38%). ^{1}H NMR (270 MHz, DMSO-d_6) δ 0.30−0.36 (2H, m), 0.54−0.61 (2H, m), 1.19−1.24 (1H, m), 1.45 (6H, s), 3.79 (2H, d, J = 6.9 Hz), 4.76 (2H, s), 5.60 (1H, d, J = 7.8 Hz), 6.97−7.06 (5H, m), 7.20−7.26 (3H, m), 7.69 (1H, d, J = 7.8 Hz), 8.02 (1H, brs), 11.32 (1H, brs). ^{13}C NMR (100 MHz, DMSO-d_6) δ 3.1, 10.0, 29.7, 49.7, 57.2, 72.4, 101.2, 111.6, 118.1, 125.8, 126.8, 129.7, 134.7, 144.4, 145.4, 145.6, 151.0, 158.3, 163.7. Anal. Calcd for $C_{24}H_{27}N_3O_5S{\cdot}0.3H_2O$: C, 60.69; H, 5.86; N, 8.85. Found: C, 60.68; H, 5.71; N, 8.96.

3-(Cyclopentyloxy)-*N*-(2-(4-((2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)methyl)phenyl)propan-2-yl)benzenesulfonamide (24). **24** was prepared from **22b** (247 mg, 0.66 mmol) as described for the preparation of **8**, pale yellow foam (22.5 mg, 0.047 mmol, 7%). ^{1}H NMR (270 MHz, DMSO-d_6) δ 1.45 (6H, s), 1.58−1.70 (6H, m), 1.89−1.95 (2H, m), 4.72−4.76 (1H, m), 4.76 (2H, s), 5.60 (1H, d, J = 7.9 Hz), 6.94−7.09 (5H, m), 7.18−7.27 (3H, m), 7.69 (1H, d, J = 7.9 Hz), 8.03 (1H, brs), 11.3 (1H, brs). ^{13}C NMR (100 MHz, DMSO-d_6) δ 23.6, 29.7, 32.2, 49.8, 57.3, 79.2, 101.3, 112.6, 118.0, 119.0, 125.8, 126.8, 129.7, 134.8, 144.4, 145.4, 145.6, 151.0, 157.4, 163.7. Anal. Calcd for $C_{25}H_{29}N_3O_5S$: C, 62.09; H, 6.04; N, 8.69. Found: C, 61.87; H, 6.04; N, 8.65.

3-(2,2-Difluoroethoxy)-*N*-(2-(4-((2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)methyl)phenyl)propan-2-yl)-benzenesulfonamide (25). **25** was prepared from **22c** (240 mg, 0.65 mmol) as described for the preparation of **8**, white foam (32.7 mg, 0.068 mmol, 10%). ^{1}H NMR (270 MHz, DMSO-d_6) δ 1.47 (6H, s), 4.35 (2H, td, J = 14.7, 3.3 Hz), 4.77 (2H, s), 5.61 (1H, d, J = 7.8 Hz), 6.41 (1H, tt, J = 54.2, 3.5 Hz), 7.02−7.17 (5H, m), 7.24−7.32 (3H, m), 7.67 (1H, d, J = 7.8 Hz), 8.06 (1H, brs), 11.32 (1H, brs). ^{13}C NMR ($CDCl_3$) δ 29.8, 50.7, 58.3, 67.3 (t, J = 24.1 Hz), 102.6, 112.4, 113.3 (t, J = 242.3 Hz), 118.9, 120.2, 126.4, 127.4, 130.0, 133.9, 143.9, 144.1, 145.1, 151.0, 157.5, 163.8. HRMS (TOF) calcd for $C_{22}H_{24}F_2N_3O_5S$ $[M + H]^+$ 480.1405, found 480.1411. HPLC purity: 96.8%, t_R = 7.65 min (method A).

(*E*)-*N*-(7-Hydroxy-2-methylhept-5-en-2-yl)-3-methoxybenzenesulfonamide (26). **26** was prepared from **17** (475 mg, 1.95 mmol) as described for the preparation of **18**, colorless oil (95.0 mg, 0.66 mmol, 34%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.19 (6H, s), 1.54−1.63 (2H, m), 2.00−2.09 (2H, m), 2.18 (3H, s), 4.49 (1H, brs), 5.52−5.57 (2H, m), 7.46−7.60 (4H, m), 7.84−7.93 (2H, m). HRMS (FAB) calcd for $C_{15}H_{22}NO_4S$ $[M - H]^-$ 312.1270, found 312.1279.

(*E*)-*N*-(7-(2,4-Dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-2-methylhept-5-en-2-yl)-3-methoxybenzenesulfonamide (27). **27** was prepared from **26** (205 mg, 0.65 mmol) as described for the preparation of **9**, white foam (35.0 mg, 0.086 mmol, 13%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.19 (6H,s), 1.61−1.66 (2H, m), 2.06−2.18 (2H, m), 3.85 (3H, s), 4.27 (2H, d, J = 6.1 Hz), 4.54 (1H, brs), 5.41−5.50 (1H, m), 5.64−5.72 (2H, m), 7.07 (1H, dd, J = 8.3, 2.7 Hz), 7.15 (1H, d, J = 8.1 Hz), 7.36−7.47 (3H, m), 8.30 (1H, brs). ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.7, 27.7, 41.6, 49.5, 55.6, 56.8, 102.3, 111.7, 118.3, 118.9, 123.3, 130.0, 136.1, 143.8, 144.6, 150.8, 159.7, 163.8. HRMS (TOF) calcd for $C_{19}H_{26}N_3O_5S$ $[M + H]^+$ 408.1593, found 408.1598. HPLC purity: 96.4%, t_R = 5.08 min (method B).

(*E*)-3-(*N*-(7-Hydroxy-2-methylhept-5-en-2-yl)sulfamoyl)-phenyl Benzoate (28). **28** was prepared from **17** (450 mg, 1.85 mmol) as described for the preparation of **18**, light brown gum (320 mg, 0.79 mmol, 43%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.22 (6H, s), 1.58−1.64 (2H, m), 2.04−2.09 (2H, m), 4.03−4.05 (2H, m), 5.07 (1H, brs), 5.57−5.62 (2H, m), 7.39−7.43 (1H, m), 7.50−7.70 (4H, m), 7.78−7.82 (2H, m), 8.17−8.21 (2H, m). HRMS (FAB) calcd for $C_{21}H_{24}NO_5S$ $[M - H]^-$ 402.1375, found 402.1406.

(*E*)-3-(Cyclopropylmethoxy)-*N*-(7-hydroxy-2-methylhept-5-en-2-yl)benzenesulfonamide (29a). **29a** was prepared from **28** (310 mg, 0.77 mmol) as described for the preparation of **22a**, colorless oil (248 mg, 0.70 mmol, 91%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.33−0.39 (2H, m), 0.62−0.70 (2H, m), 1.20 (6H, s), 1.22−1.30 (1H, m), 1.59−1.64 (2H, m), 2.01−2.10 (2H, m), 3.84 (2H, d, J = 7.0 Hz), 4.07 (2H, d, J = 4.1 Hz), 4.42 (1H, brs), 5.60−5.64 (2H, m), 7.05−7.09 (1H, m), 7.35−7.47 (3H, m). HRMS (FAB) calcd for $C_{18}H_{26}NO_4S$ $[M - H]^-$ 352.1583, found 352.1587.

(*E*)-3-(Cyclopentyloxy)-*N*-(7-hydroxy-2-methylhept-5-en-2-yl)benzenesulfonamide (29b). **29b** was prepared from **28** (600 mg, 1.49 mmol) as described for the preparation of **22a**, yellow oil (292 mg, 0.79 mmol, 53%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.20 (6H, s), 1.59−1.64 (2H, m), 1.75−2.00 (8H, m), 2.05−2.10 (2H, m), 4.05−4.09 (2H, m), 4.19 (1H, brs), 4.40−4.44 (1H, m), 5.61−5.64 (2H, m), 7.00−7.04 (1H, m), 7.35−7.47 (3H, m). HRMS (FAB) calcd for $C_{19}H_{28}NO_4S$ $[M - H]^-$ 366.1739, found 366.1762.

(*E*)-3-(2,2-Difluoroethoxy)-*N*-(7-hydroxy-2-methylhept-5-en-2-yl)benzenesulfonamide (29c). **29c** was prepared from **28** (600 mg, 1.49 mmol) as described for the preparation of **22a**, yellow oil (426 mg, 1.17 mmol, 79%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.20 (6H, s), 1.59−1.64 (2H, m), 2.01−2.17 (2H, m), 4.05−4.09 (2H, m), 4.23 (2H, td, J = 12.9, 4.2 Hz), 4.49 (1H, brs), 5.61−5.64 (2H, m), 6.11 (1H, tt, J = 55.1, 3.9 Hz), 7.08−7.11 (1H, m), 7.37−7.45 (3H, m). HRMS (FAB) calcd for $C_{16}H_{22}F_2NO_4S$ $[M - H]^-$ 362.1238, found 362.1269.

(*E*)-3-(Cyclopropylmethoxy)-*N*-(7-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-2-methylhept-5-en-2-yl)benzenesulfonamide (30). **30** was prepared from **29a** (248 mg, 0.70 mmol) as described for the preparation of **9**, colorless gum (108 mg, 0.24 mmol, 34%). ^{1}H NMR (270 MHz, DMSO-d_6) δ 0.30−0.34 (2H, m), 0.53−0.56 (2H, m), 1.01 (6H, s), 1.15−1.24 (1H, m), 1.39−1.45 (2H, m), 1.91−1.95 (2H, m), 3.85 (2H, d, J = 6.9 Hz), 4.16 (2H, d, J = 5.1 Hz), 5.30−5.58 (2H, m), 5.60 (1H, d, J = 7.8 Hz), 7.09−7.13 (1H, m), 7.30−7.45 (4H, m), 7.64 (1H, d, J = 7.8 Hz), 11.30 (1H, brs). ^{13}C NMR (100 MHz, $CDCl_3$) δ 3.2, 10.0, 26.7, 27.6, 41.6, 49.5, 56.8, 73.1, 102.2, 112.2, 118.8, 119.0, 123.3, 129.9, 136.1, 143.8, 144.5, 150.8, 159.1,

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163.9. HRMS (TOF) calcd for $C_{22}H_{30}N_3O_5S$ $[M + H]^+$ 448.1906, found 448.1909. HPLC purity: 95.1%, t_R = 6.05 min (method B).

(*E*)-3-(Cyclopentyloxy)-*N*-(7-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-2-methylhept-5-en-2-yl)benzenesulfonamide (31). **31** was prepared from **29b** (125 mg, 0.34 mmol) as described for the preparation of **9**, colorless gum (24.5 mg, 0.053 mmol, 16%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.19 (6H, s), 1.58−1.70 (4H, m), 1.74−1.88 (4H, m), 1.89−1.97 (2H, m), 2.07−2.15 (2H, m), 4.27 (2H, d, J = 6.3 Hz), 4.74 (1H, brs), 4.77−4.82 (1H, m), 5.41−5.51 (1H, m), 5.64−5.70 (1H, m), 5.72 (1H, d, J = 7.7 Hz), 7.02 (1H, dd, J = 8.1, 1.5 Hz), 7.16 (1H, d, J = 7.7 Hz), 7.33−7.45 (3H, m), 8.69 (1H, brs). ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.0, 26.8, 27.7, 32.7, 41.6, 49.6, 56.8, 79.9, 102.3, 113.3, 118.5, 119.9, 123.3, 130.0, 136.2, 143.7, 144.3, 150.6, 158.3, 163.5. Anal. Calcd for $C_{23}H_{31}N_3O_5S\cdot0.9H_2O$: C, 57.82; H, 6.92; N, 8.79. Found: C, 57.46; H, 6.53; N, 8.42.

(*E*)-3-(2,2-Difluoroethoxy)-*N*-(7-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-2-methylhept-5-en-2-yl)benzenesulfonamide (32). **32** was prepared from **29c** (110 mg, 0.30 mmol) as described for the preparation of **9**, colorless gum (23.0 mg, 0.050 mmol, 17%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.19 (6H, s), 1.58−1.70 (2H, m), 2.06−2.17 (2H, m), 4.23−4.28 (4H, m), 4.63 (1H, brs), 5.41−5.51 (1H, m), 5.64−5.70 (2H, m), 6.11 (1H, tt, J = 54.8, 3.8 Hz), 7.09−7.17 (2H, m), 7.40−7.55 (3H, m), 8.40 (1H, brs). ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.8, 27.7, 41.5, 49.7, 57.0, 67.4 (t, J = 24.0 Hz), 102.3, 112.6, 113.3 (t, J = 240.7 Hz), 118.9, 120.2, 123.4, 130.3, 135.9, 143.8, 144.9, 150.7, 157.8, 163.6. Anal. Calcd for $C_{20}H_{25}F_2N_3O_5S\cdot H_2O$: C, 50.52; H, 5.72; N, 8.84. Found: C, 50.74; H, 5.40; N, 8.60.

(*R*)-4-(Bromomethyl)-*N*-(1-(3-(cyclopropylmethoxy)phenyl)-ethyl)benzenesulfonamide (37a). To a stirred solution of (*R*)-1-(3-(cyclopropylmethoxy)phenyl)ethanamine hydrochloride **36a** (363 mg, 1.60 mmol) in CH_2Cl_2 (3.0 mL) were added Et_3N (670 μL, 4.81 mmol) and 4-(bromomethyl)benzenesulfonyl chloride (450 mg, 1.67 mmol) at 0 °C, and the resultant was stirred at the same temperature for 1 h. The mixture was poured into H_2O and extracted with EtOAc. The organic layer was washed with 1 N HCl, brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel, eluting with hexane/EtOAc = 3/1 to afford the title compound (188 mg, 0.44 mmol, 28%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.31−0.34 (2H, m), 0.63−0.65 (2H, m), 1.19−1.28 (1H, m), 1.43 (3H, d, J = 6.8 Hz), 3.66−3.70 (2H, m), 4.48 (1H, quin, J = 7.8 Hz), 4.57 (2H, s), 4.69 (1H, brs), 6.60−6.64 (2H, m), 6.68−6.71 (1H, m), 7.08 (1H, td, J = 7.6, 1.2 Hz), 7.37−7.40 (2H, m), 7.68−7.71 (2H, m). HRMS (FAB) calcd for $C_{19}H_{21}BrNO_3S$ $[M - H]^-$ 422.0426, found 422.0414.

(*R*)-4-(Bromomethyl)-*N*-(1-(3-(cyclopentyloxy)phenyl)ethyl)-benzenesulfonamide (37b). **37b** was prepared from **36b** (387 mg, 1.60 mmol) as described for the preparation of **37a**, white solid (419 mg, 0.96 mmol, 60%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.44 (3H, t, J = 6.6 Hz), 1.58−1.64 (2H, m), 1.72−1.89 (6H, m), 4.43 (2H, s), 4.46 (1H, quin, J = 6.8 Hz), 4.63 (1H, brs), 4.69 (1H, d, J = 7.1 Hz), 6.57−6.61 (2H, m), 6.66−6.70 (1H, m), 7.05 (1H, t, J = 8.3 Hz), 7.38 (2H, dd, J = 8.3, 2.6 Hz), 7.66−7.70 (2H, m). HRMS (FAB) calcd for $C_{20}H_{23}BrNO_3S$ $[M - H]^-$ 436.0582, found 436.0585.

(*R*)-4-(Bromomethyl)-*N*-(1-(3-(2,2-difluoroethoxy)phenyl)-ethyl)benzenesulfonamide (37c). **37c** was prepared from **36c** (380 mg, 1.60 mmol) as described for the preparation of **37a**, colorless oil (337 mg, 0.78 mmol, 49%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.43 (3H, d, J = 6.9 Hz), 3.97−4.14 (2H, m), 4.44−4.52 (1H, m), 4.56 (2H, s), 4.87 (1H, d, J = 6.9 Hz), 6.04 (1H, tt, J = 57.0, 4.0 Hz), 6.59−6.61 (1H, m), 6.69−6.77 (2H, m), 7.13 (1H, t, J = 7.9 Hz), 7.36−7.40 (2H, m), 7.64−7.69 (2H, m). HRMS (FAB) calcd for $C_{17}H_{17}BrF_2NO_3S$ $[M - H]^-$ 432.0081, found 432.0077.

(*R*)-*N*-(1-(3-(Cyclopropylmethoxy)phenyl)ethyl)-4-((2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)methyl)-benzenesulfonamide (38). To a suspension of 2,4-bis-(trimethylsilyloxy)pyrimidine (130 mg, 0.51 mmol) in 1,2-dichloroethane (2.5 mL) was added a solution of **37a** (143 mg, 0.34 mmol) in 1,2-dichloroethane (1.0 mL) and iodine (catalyst) at room temperature. The resulting mixture was heated to reflux at 95 °C for 5 h. After cooling to room temperature, the mixture was poured into H_2O/saturated aqueous $Na_2S_2O_3$ and extracted with EtOAc two times. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel, eluting with EtOAc to afford the title compound (107 mg, 0.24 mmol, 68%) as a colorless gum. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.29−0.35 (2H, m), 0.60−0.67 (2H, m), 1.17−1.28 (1H, m), 1.42 (3H, d, J = 7.1 Hz), 3.69 (2H, dd, J = 7.1, 2.4 Hz), 4.46 (1H, quin, J = 7.1 Hz), 4.91 (2H, s), 5.16 (1H, brs), 5.76 (1H, d, J = 7.9, 2.0 Hz), 6.59−6.68 (3H, m), 7.04 (1H, t, J = 8.2 Hz), 7.14 (1H, d, J = 7.9 Hz), 7.23−7.28 (2H, m), 7.69 (2H, d, J = 8.2 Hz), 8.95 (1H, brs). ^{13}C NMR (100 MHz, DMSO-d_6) δ 3.1, 10.2, 23.5, 49.9, 53.0, 71.8, 101.5, 112.2, 112.7, 118.1, 126.7, 127.7, 129.0, 140.8, 144.7, 145.4, 145.6, 151.0, 158.4, 163.7. Anal. Calcd for $C_{23}H_{25}N_3O_5S\cdot0.3H_2O$: C, 59.93; H, 5.60; N, 9.12. Found: C, 60.05; H, 5.28; N, 9.01. $[\alpha]^{25}_D$ +41.4 (c 0.20, MeOH).

(*R*)-*N*-(1-(3-(Cyclopentyloxy)phenyl)ethyl)-4-((2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)methyl)benzenesulfonamide (39). **39** was prepared from **37b** (191 mg, 0.44 mmol) as described for the preparation of **38**, colorless gum (50 mg, 0.11 mmol, 25%). ^{1}H NMR (270 MHz, DMSO-d_6) δ 1.18 (3H, d, J = 7.0 Hz), 1.54−1.64 (6H, m), 1.83−2.10 (2H, m), 3.25−3.43 (1H, m), 4.26−4.29 (1H, m), 4.62−4.70 (1H, m), 4.87 (2H, s), 5.62 (1H, d, J = 8.2 Hz), 6.56−6.53 (2H, m), 6.70 (1H, s), 6.97 (1H, t, J = 7.8 Hz), 7.29−7.33 (2H, m), 7.58−7.62 (2H, m), 8.16 (1H, d, J = 8.2 Hz), 8.30 (1H, brs). ^{13}C NMR (100 MHz, DMSO-d_6) δ 23.5, 23.6, 32.2, 49.7, 53.0, 78.4, 101.5, 113.2, 113.5, 117.9, 126.7, 127.6, 129.0, 140.8, 144.6, 145.4, 145.6, 151.0, 157.4, 163.7. Anal. Calcd for $C_{24}H_{27}N_3O_5S\cdot0.5H_2O$: C, 60.23; H, 5.90; N, 8.78. Found: C, 60.32; H, 5.65; N, 8.49. $[\alpha]^{25}_D$ +33.4 (c 0.45, MeOH).

(*R*)-*N*-(1-(3-(2,2-Difluoroethoxy)phenyl)ethyl)-4-((2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)methyl)benzenesulfonamide (40). **40** was prepared from **37c** (158 mg, 0.36 mmol) as described for the preparation of **38**, colorless gum (84.6 mg, 0.18 mmol, 50%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.44 (3H, d, J = 6.8 Hz), 4,00−4.10 (2H, m), 4.48−4.54 (1H, m), 4.87 (1H, d, J = 6.8 Hz), 4.91(2H, s), 5.76 (1H, dd, J = 8.1, 1.7 Hz), 6.05 (1H, tt, J = 55.4, 4.1 Hz), 6.57 (1H, s), 6.69 (1H, dd, J = 8.3, 2.7 Hz), 6.73−6.77 (1H, m), 7.09−7.15 (2H, m), 7.24−7.28 (1H, m), 7.67 (3H, d, J = 8.3 Hz), 8.34 (1H, brs). ^{13}C NMR (100 MHz, DMSO-d_6) δ 23.6, 49.9, 52.8, 66.2 (t, J = 24.0 Hz), 101.6, 112.4, 112.7, 114.1 (t, J = 242 Hz), 119.2, 126.7, 127.6, 129.2, 140.6, 140.9, 145.0, 145.6, 151.0, 157.3, 163.7. Anal. Calcd for $C_{21}H_{21}F_2N_3O_5S$: C, 53.16; H, 4.67; N, 8.86. Found: C, 53.34; H, 4.32; N, 8.77. $[\alpha]^{25}_D$ +38.8 (c 0.50, MeOH).

(*R*,*E*)-5-(3-Benzoyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-*N*-(1-(3-(cyclopropylmethoxy)phenyl)ethyl)-*N*-(methoxymethyl)pent-3-ene-1-sulfonamide (42a). To a solution of **41a** (3.60 g, 9.39 mmol) in THF (60 mL) were added *N*-3-benzoyluracil (3.10 g, 14.3 mmol) and PPh_3 (3.91 g, 14.9 mmol) at room temperature. To the mixture was slowly added a solution of DEAD in toluene (2.2 M, 6.41 mL, 14.1 mmol) in THF (2.0 mL), and the whole mixture was stirred at room temperature for 2 h. The mixture was concentrated under reduced pressure, and the concentrate was purified by column chromatography on silica gel, eluting with hexane/EtOAc = 1/1 to afford the title compound (4.92 g, 8.46 mmol, 90%) as a colorless gum. ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.33−0.37 (2H, m), 0.63−0.67 (2H, m), 1.22−1.32 (1H, m), 1.66 (3H, d, J = 7.3 Hz), 2.58−2.66 (2H, m), 3.04−3.12 (2H, m), 3.24 (3H, s), 3.80 (2H, d, J = 7.0 Hz), 4.28−4.34 (3H, m), 4.68 (1H, d, J = 10.5 Hz), 5.09−5.13 (1H, m), 5.53−5.69 (1H, m), 5.76−5.83 (2H, m), 6.82−6.88 (1H, m), 6.95−6.99 (2H, m), 7.22−7.64 (5H, m), 7.92−7.96 (2H, m). HRMS (FAB) calcd for $C_{30}H_{35}N_3NaO_7S$ $[M + Na]^+$ 604.2093, found 604.2127.

(*R*,*E*)-5-(3-Benzoyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-*N*-(1-(3-(cyclopentyloxy)phenyl)ethyl)-*N*-(methoxymethyl)-pent-3-ene-1-sulfonamide (42b). **42b** was prepared from **41b** (306 mg, 0.77 mmol) as described for the preparation of **42a**, colorless gum (420 mg, 0.71 mmol, 92%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.53−1.68 (5H, m), 1.75−1.94 (6H, m), 2.59−2.67 (2H, m), 3.03−3.15 (2H, m), 3.24 (3H, s), 4.32−4.37 (3H, m), 4.68 (1H, d, J = 10.6 Hz), 4.74−4.78 (1H, m), 5.10 (1H, q, J = 7.3 Hz), 5.56−5.67 (1H, m), 5.77−5.81 (2H, m), 6.78−6.82 (1H, m), 6.93−6.95 (2H, m), 7.21−7.26 (1H, m),

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7.31 (1H, d, J = 8.1 Hz), 7.43−7.56 (2H, m), 7.62−7.70 (1H, m), 7.92−7.96 (2H, m). HRMS (FAB) calcd for $C_{31}H_{37}N_3NaO_7S$ $[M + Na]^+$ 618.2250, found 618.2248.

(*R*,*E*)-5-(3-Benzoyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-*N*-(1-(3-(2,2-difluoroethoxy)phenyl)ethyl)-*N*-(methoxymethyl)-pent-3-ene-1-sulfonamide (42c). **42c** was prepared from **41c** (173 mg, 0.44 mmol) as described for the preparation of **42a**, colorless gum (211 mg, 0.36 mmol, 81%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.67 (3H, d, J = 7.3 Hz), 2.60−2.68 (2H, m), 3.03−3.12 (2H, m), 3.25 (3H, s), 4.19 (2H, td, J = 13.2, 4.3 Hz), 4.28−4.36 (3H, m), 4.69 (1H, d, J = 10.6 Hz), 5.09−5.14 (1H, m), 5.57−5.67 (1H, m), 5.77−5.90 (2H, m), 6.09 (1H, tt, J = 55.1, 4.1 Hz), 6.85 (1H, dd, J = 8.6, 3.0 Hz), 7.01 (1H, s), 7.07 (1H, d, J = 7.8 Hz), 7.25−7.29 (2H, m), 7.43−7.56 (2H, m), 7.62−7.70 (1H, m), 7.92−7.96 (2H, m). HRMS (FAB) calcd for $C_{28}H_{31}F_2N_3NaO_7S$ $[M + Na]^+$ 614.1748, found 614.1741.

(*R*,*E*)-*N*-(1-(3-(Cyclopropylmethoxy)phenyl)ethyl)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)pent-3-ene-1-sulfonamide (43). A solution of **42a** (4.90 g, 8.42 mmol) in 40% $MeNH_2$ in MeOH (100 mL) was stirred at room temperature for 1 h. The mixture was concentrated under reduced pressure, and the residue was coevaporated with toluene two times. This residue was dissolved in 4 N HCl/dioxane (30.0 mL). The resulting mixture was stirred at room temperature for 4 h and then poured into saturated aqueous $NaHCO_3$. The aqueous layer was extracted with $CHCl_3$/MeOH (10/1) three times. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel, eluting with $CHCl_3$/EtOAc = 3/1 to afford the title compound (2.73 g, 6.30 mmol, 75% from **42a**) as a colorless gum. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.33−0.37 (2H, m), 0.62−0.68 (2H, m), 1.22−1.31 (1H, m), 1.53 (3H, d, J = 7.0 Hz), 2.36−2.47 (2H, m), 2.66−2.73 (1H, m), 2.79−2.86 (1H, m), 3.80 (2H, d, J = 6.8 Hz), 4.20−4.29 (2H, m), 4.59 (1H, quin, J = 7.0 Hz), 4.66 (1H, brs), 5.42−5.49 (1H, m), 5.54−5.61 (1H, m), 5.71 (1H, dd, J = 7.8, 2.0 Hz), 6.80−6.83 (1H, m), 6.86−6.92 (2H, m), 7.12 (1H, d, J = 7.8 Hz), 7.24−7.29 (1H, m), 8.25 (1H, brs). ^{13}C NMR (100 MHz, $CDCl_3$) δ 3.2, 10.2, 24.0, 26.5, 49.3, 52.5, 53.7, 72.8, 102.4, 112.8, 113.6, 118.4, 125.6, 129.9, 132.1, 143.8, 144.3, 150.7, 159.4, 163.7. Anal. Calcd for $C_{21}H_{27}N_3O_5S$: C, 58.18; H, 6.28; N, 9.69. Found: C, 57.89; H, 6.34; N, 9.53. $[\alpha]^{25}_D$ +17.4 (c 0.42, MeOH).

(*R*,*E*)-*N*-(1-(3-(Cyclopentyloxy)phenyl)ethyl)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)pent-3-ene-1-sulfonamide (44). **44** was prepared from **42b** (420 mg, 0.71 mmol) as described for the preparation of **43**, colorless gum (89.0 mg, 0.20 mmol, 28%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.53 (3H, d, J = 6.8 Hz), 1.61−1.71 (2H, m), 1.77−1.93 (6H, m), 2.33−2.48 (2H, m), 2.69−2.85 (2H, m), 4.22−4.25 (2H, m), 4.51−4.58 (2H, m), 4.75−4.79 (1H, m), 5.47−5.68 (2H, m), 5.70 (1H, dd, J = 7.8, 2.2 Hz), 6.74−6.87 (3H, m), 7.12 (1H, d, J = 8.0 Hz), 7.21−7.26 (1H, m), 8.28 (1H, brs). ^{13}C NMR (100 MHz, DMSO-d_6) δ 23.5, 24.1, 26.0, 32.2, 48.1, 51.2, 52.8, 78.4, 101.1, 113.3, 113.8, 118.0, 125.9, 129.4, 130.5, 145.0, 145.6, 150.7, 157.7, 163.7. Anal. Calcd for $C_{22}H_{29}N_3O_5S{\cdot}0.5H_2O$: C, 57.88; H, 6.62; N, 9.20. Found: C, 57.76; H, 6.33; N, 9.14. $[\alpha]^{25}_D$ +17.9 (c 0.48, MeOH).

(*R*,*E*)-*N*-(1-(3-(2,2-Difluoroethoxy)phenyl)ethyl)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)pent-3-ene-1-sulfonamide (45). **45** was prepared from **42c** (210 mg, 0.35 mmol) as described for the preparation of **43**, colorless gum (70.0 mg, 0.16 mmol, 46%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.54 (3H, d, J = 6.9 Hz), 2.36−2.48 (2H, m), 2.68−2.87 (2H, m), 4.14−4.25 (4H, m), 4.61 (1H, quin, J = 6.9 Hz), 4.94 (1H, brs), 5.42−5.60 (2H, m), 5.72 (1H, d, J = 6.5 Hz), 6.10 (1H, tt, J = 55.1 Hz, 4.1 Hz), 6.81−7.06 (3H, m), 7.13 (1H, d, J = 7.9 Hz), 7.26−7.31 (1H, m), 8.64 (1H, brs). ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.0, 26.5, 49.4, 52.5, 53.5, 67.2 (t, J = 24.1 Hz), 102.4, 112.8, 113.5, 113.6 (t, J = 244.0 Hz), 119.8, 125.6, 130.2, 131.8, 144.0, 144.8, 150.8, 158.1, 163.9. HRMS (TOF) calcd for $C_{19}H_{24}F_2N_3O_5S$ $[M + H]^+$ 444.1405, found 444.1412. HPLC purity: 96.2%, t_R = 4.90 min (method B). $[\alpha]^{25}_D$ +16.4 (c 0.22, MeOH).

Molecular Modeling Studies. All molecular modeling was performed using the Molecular Operating Environment (MOE, version 2010.10), developed by Chemical Computing Group, Inc. (Montreal, Canada). Ligand structures were built using MOE builder tool, part of the MOE suite, and were subjected to MMFF94x energy minimization until rmsd gradient was <0.05 kcal mol^{-1} Å^{-1}.

Hydrogen atoms were added to the X-ray cocrystal structure of dUTPase and compound **7** (PDB code 3ARN[35]), and the energy of the structure was minimized keeping fixed the atoms of the mainframe. The models **8** and **9** were constructed on the basis of crystal structure conformation of compound **7** followed by energy minimization. A part of the flexible linker (-O-CH_2-CH_2-CH_2-) of compound **7** has enough planarity to perform the modeling (dihedral angle is 35°). Molecular graphics were prepared using the PyMOL Molecular Graphics System, version 1.2r3pre (Schrödinger, LLC).

Cloning, Expression, and Purification of Recombinant Human dUTPase. The cDNA of human dUTPase was subcloned into the expression vector pET19b. The construct was then transformed into *E. coli* BL21(DE3) cells (Novagen) in Luria broth at 37 °C. Protein expression was induced with 0.01 mM isopropyl-β-D-thiogalactopyranoside (IPTG) at an optical density of 0.6 at 595 nm. The cell pellet was resuspended in ice-cold lysis buffer containing 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, and 1 mM dithiothreitol (DTT). After sonication, the disrupted debris was removed by centrifugation. The supernatant was applied to Ni-NTA affinity gels, and the 6× His-Tag was removed by digestion with enterokinase in 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, and 1 mM DTT for 12 h. The protein solutions used for crystallization were gel-filtered in a buffer containing 50 mM Tris-HCl, pH 7.5, 50 mM NaCl, and 1 mM DTT, on a preparative grade Superdex 75 column (GE Healthcare Life Sciences).

dUTPase Inhibition Assay. In vitro dUTPase inhibition assays were conducted by measuring the production of [5-^{3}H]dUMP from [5-^{3}H]dUTP. Breifly, 0.2 mL of a solution containing 0.02 mL of 1 μM dUTP (including 588 Bq/mL [5-^{3}H]dUTP), 0.05 mL of 0.2 M Tris buffer solution (pH 7.4), 0.05 mL of 16 mM magnesium chloride, 0.02 mL of 20 mM 2-mercaptoethanol, 0.02 mL of a 1% aqueous solution of fetal bovine serum-derived albumin, 0.02 mL of varying concentrations of test compound solutions or pure water as a control, and 0.02 mL of a solution of human dUTPase was reacted at 37 °C for 15 min. After the reaction, the solution was immediately heated at 100 °C for 1 min to terminate the reaction and then centrifuged at 15 000 rpm for 2 min. An aliquot (150 μL) of the supernatant thus obtained by centrifugation was analyzed using an Atlantis C18 column (manufactured by Waters Corp., 4.6 mm × 250 mm) and a high-performance liquid chromatograph (manufactured by Shimadzu Corp., Prominence). The inhibition rate of the compound was determined according to the formula shown below.

$$\text{inhibition rate (\%)} = 1 - [(\text{amount of [5-}^3\text{H]dUMP in the presence of test solution (dpm)})/(\text{amount of [5-}^3\text{H]dUMP as control (dpm))}] \times 100$$

IC_{50} (μM), the concentration of inhibitor yielding 50% inhibition rate, was obtained from the concentration−inhibition rate curve.

In Vitro Cell Inhibition Assays. HeLa S3 (human cervix adenocarcinoma) cells were cultured in RPMI-1640 supplemented with 10% FBS. Exponentially growing cells were seeded in 96-well plates (1500 cells/0.18 mL) and incubated at 37 °C in a humidified 5% CO_2 atmosphere for 24 h. Vehicle-control (DMSO) and test compounds (1−100 μM) were added to the plates at 20 μL per well, and the plates were incubated for 72 h. Cell proliferation was determined by the crystal violet assay. Optical density at 540 nm (OD_{540}) was measured by plate reader. Then we calculated T/C (%), which is the ratio of OD_{540} with drug treatment to OD_{540} without drug: T/C (%) = [(OD_{540} of treated well)/(OD_{540} of nontreated well)] × 100. The IC_{50} (μM) for the cytotoxicity of the test compound is the concentration yielding 50% T/C, which was calculated from concentration−T/C (%) curve.

In Vitro Cell Inhibition Assays in Combination with FdUrd. HeLa S3 cells were seeded in 96-well plates (1500 cells/0.18 mL) and

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incubated at 37 °C in a humidified 5% CO_2 atmosphere as described above. After 24 h, vehicle-control (DMSO) and test compounds (1−100 μM) in combination with FdUrd (1 μM) were added to the plates at a volume of 20 μL per well and incubated for 24 h. Then thymidine (30 μM) was added to the plates at a volume of 10 μL per well and incubated for 48 h. Cell proliferation was determined by the crystal violet assay as mentioned above. The EC_{50} was calculated from the concentration− T/C (%) curve as a concentration of each compound that reduces the T/C (%) of FdUrd (1 μM) against HeLa S3 cells to half in 24 h.

Pharmacokinetic Studies. Experiments were conducted with 6-week-old to 9-week-old male Balb/c-A mice. Compound **43** was administered to mice orally at a dose of 50 mg/kg in a solution containing 2.5% DMA/2.5% Tween 80 and 10% Cremophor EL. The concentration of **43** in the plasma was determined by ultraperformance liquid chromatography (UPLC).

Evaluation of Antitumor Efficacy of Compound 43. Five-week-old Balb/cA JcL-nu mice were obtained from Clea Japan, Inc. (Tokyo, Japan). MX-1 human breast carcinoma (Japanese Foundation for Cancer Research) was maintained by subcutaneous (sc) transplantation in mice. Briefly, tumors were excised and fragments (approximately 2 mm in diameter) were implanted sc using a trocar. After implantation, the animals were divided into four groups and treated either with vehicle (2.5% DMA, 2.5% Tween 80, 10% Cremophor, and 0.5% HPMC), 5-FU (15 mg kg^{-1} day^{-1}) by continuous infusion using osmotic pump for 14 days, and compound **43** (300 mg kg^{-1} mg^{-1}) by po for 14 days or with a combination of 5-FU (15 mg kg^{-1} day^{-1}) and compound **43** (300 mg kg^{-1} mg^{-1}). Tumor size and body weight were measured twice weekly. Tumor volume (TV) was estimated with the formula TV (mm^3) = length (mm) × width (mm) × width (mm) × 0.5. Relative tumor volume (RTV) on day 15 was calculated as the ratio of TV on day 15 to that on day 0 according to the following formula: RTV = (TV on day 15)/(TV on day 0). Body weight change (%) on day 15 was calculated according to the following formula: body weight change (%) = [(body weight on day 15) − (body weight on day 0)]/(body weight on day 0) × 100.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Synthetic procedures and characterization data for compounds **36b−c** and **41a−c**. This material is available free of charge via the Internet at http://pubs.acs.org.

Accession Codes

The PDB code of compound **7** with human dUTPase is 3ARN.[36]

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

dUTPase, deoxyuridine triphosphatase; dUTP, 2′-deoxyuridine 5′-triphosphate; dTTP, thymidine 5′-triphosphate; dUMP, 2′-deoxyuridine 5′-monophosphate; TS, thymidylate synthase; 5-FU, 5-fluorouracil; FdUrd, 5-fluoro-2′-deoxyuridine; FdUTP, 5-fluoro-2′-deoxyuridine 5′-triphosphate; HCC, hepatocelluar carcinoma; SAR, structure−activity relationship

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