Letter

Design, Synthesis, and Bioevaluation of Pyrido[2,3-d]pyrimidin-7-ones as Potent SOS1 Inhibitors

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ABSTRACT: The use of small molecular modulators to target the guanine nucleotide exchange factor SOS1 has been demonstrated to be a promising strategy for the treatment of various KRAS-driven cancers. In the present study, we designed and synthesized a series of new SOS1 inhibitors with the pyrido[2,3-d]pyrimidin-7-one scaffold. One representative compound **8u** showed comparable activities to the reported SOS1 inhibitor BI-3406 in both the biochemical assay and the 3-D cell growth inhibition assay. Compound **8u** obtained good cellular activities against a panel of KRAS G12-mutated cancer cell lines and inhibited downstream ERK and AKT activation in MIA PaCa-2 and AsPC-1 cells. In addition, it displayed synergistic antiproliferative effects when used in combination with KRAS G12C or G12D inhibitors. Further modifications of the new compounds may give us a promising SOS1 inhibitor with favorable druglike properties for use in the treatment of KRAS-mutated patients.
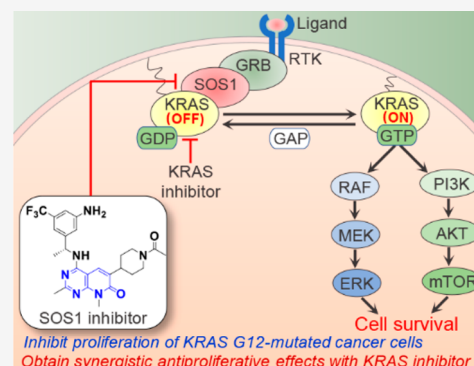
KRAS protein acts as a critical molecular switch cyclizing between the GTP-bound active state and GDP-bound
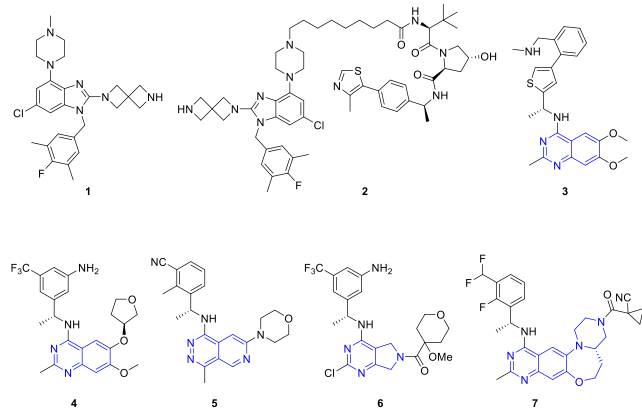

Figure 1. Representative SOS1 activator **1**, degrader **2**, and inhibitors **3-7**.

inactive state in cell signal transduction. This process is regulated by two classes of regulatory factors: guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs). GEFs help KRAS release GDP and cycle to the GTP-bound active state, and GAPs can accelerate the hydrolysis of GTP to GDP.[1] KRAS mutations are frequently detected in human cancers, especially the pancreatic cancer,
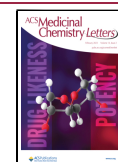
nonsmall cell lung cancer, and colorectal cancer.[2] The KRAS mutations in human cancers mainly occur at Gly12, with the top 3 mutations being G12D, G12V, and G12C.[3] These mutations inhibit the intrinsic KRAS GTP-hydrolase activity and impair the activity of GAPs,[4] thereby resulting in aberrantly activated downstream pathways and subsequently uncontrolled cancer cell proliferation.[5] It is, thus, attractive to develop drugs regulating KRAS mutant status for cancer treatment.
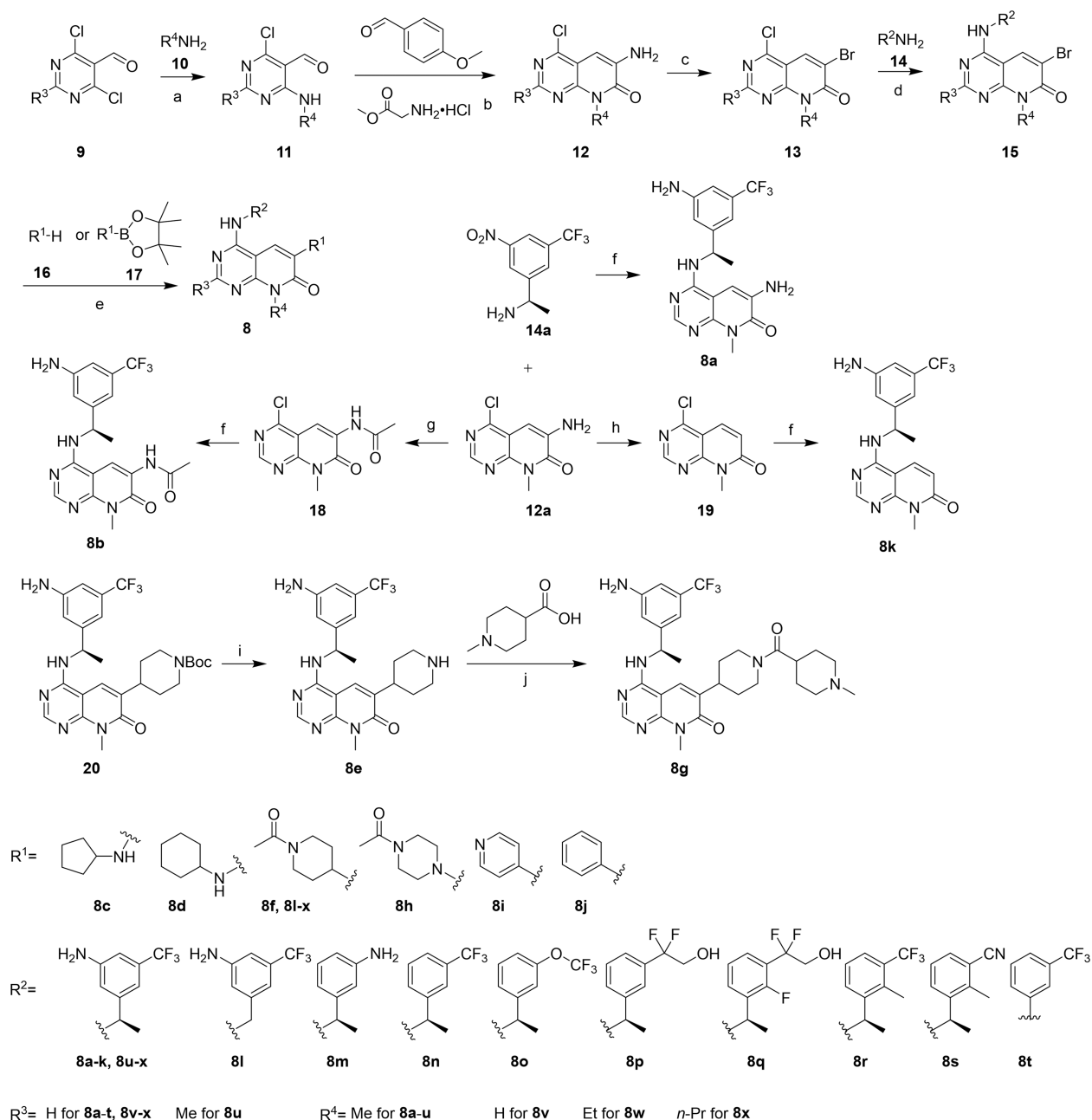
Over the past ten years, great progress has been made with drugs directly targeting KRAS mutations. This is highlighted by the approval of sotorasib, which was designed to specially target KRAS[G12C].[6] More recently, small molecules selectively targeting G12D,[7] G12S,[8] and G12R[9]-mutated KRAS have also been reported, which may lead to the development of new isoform-specific drugs. However, acquired drug resistance to the KRAS G12C inhibitors sotorasib[6] and adagrasib[10] has emerged in patients. Some putative resistance mechanisms, including acquired KRAS alterations and activation of compensatory bypass pathways, have been reported.[10] Therefore, the design of new molecules targeting the upstream
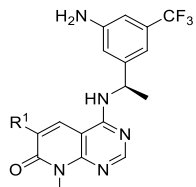
Scheme 1. Synthesis of Pyrido[2,3-*d*]pyrimidin-7-ones 8[a]

R^3= H for **8a-t, 8v-x**   Me for **8u** R^4= Me for **8a-u**   H for **8v**   Et for **8w**   *n*-Pr for **8x**
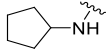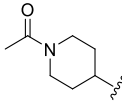
[a]Reagents and conditions: (a) CHCl₃, Et₃N, 0 °C; rt, overnight. (b) Two steps: (1) Et₃N, MeOH, rt, overnight; (2) 70% AcOH, 50 °C, 8 h. (c) CuBr, NaBr, *t*-BuONO, CH₃CN, 0 °C; rt, overnight. (d) DIPEA, CsF, DMSO, 80 °C, overnight; for **8t**, TFA, *i*-PrOH, 95 °C, overnight. (e) Two steps: (1) for **8c,d** and **8h** = amines **16**, Pd(AcO)₂, xantphos, Cs₂CO₃, 1,4-dioxane, 90 °C, overnight; for **8f, 8i,j, 8l−x**, and **20** = reagents **17**, Cs₂CO₃, Pd(dppf)Cl₂, 1,4-dioxane, 80 °C, overnight; (2) for **8c,d, 8j, 8l−x**, and **20** = Pd/C, H₂, EtOAc, rt, overnight; for **8h,i** = Fe, NH₄Cl, EtOH, 90 °C, overnight. (f) Two steps: (1) **14a**, DIPEA, CsF, DMSO, 80 °C, overnight; (2) Pd/C, H₂, EtOAc, rt, overnight. (g) AcCl, DIPEA, CH₃CN, reflux, overnight. (h) HCl (conc.), urea, NaNO₂, H₃PO₂, THF, 0 °C, 5 h. (i) HCl (4 M in 1,4-dioxane), DCM, 0 °C; rt, overnight. (j) HATU, DIPEA, DMF, rt, overnight.
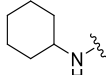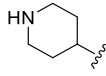
regulators or downstream effectors of KRAS may provide an alternative solution to overcome certain types of drug resistance and hopefully bring in more benefits for patients. Among these regulators/effectors, Son of Sevenless 1 (SOS1)—the primary KRAS GEF[11]—has been shown to be a promising target. The essential role of SOS1 is to regulate the nucleotide exchange of KRAS from GDP-bound inactive statu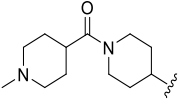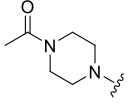s to its active status. This function is not compromised in a range of KRAS variants, including the G12C mutation, regardless of the predominant activation state of KRAS in these variants.[4,12] Deletion of SOS1 showed growth inhibition in a broad panel of KRAS-driven cancer cells.[13,14]

Small molecules targeting SOS1 have been explored in recent studies (Figure 1). For example, Fesik's group reported several series of SOS1 activators exemplified by compound **1**.[15] The activators could increase guanine nucleotide exchange[16] and elicit the biphasic modulation of downstream ERK phosphorylation.[15] On the basis of compound **1**, our group

Table 1. Structures and Biochemical Activities of 8a−k with Different R¹

Compound	R¹	SOS1 HTRF IC$_{50}$ (nM)[a]
8a	NH$_2$	> 5000
8b	(acetamido group)	> 5000
8c	(cyclopentylamino group)	463 ± 92
8d	(cyclohexylamino group)	> 5000
8e	(piperidin-4-yl group)	94.0 ± 4.8
8f	(1-acetylpiperidin-4-yl group)	87.8 ± 9.2
8g	(1-methylpiperidine-4-carbonyl piperidine group)	171 ± 31
8h	(4-acetylpiperazin-1-yl group)	72.1 ± 8.8
8i	(pyridin-4-yl group)	70.5 ± 3.9
8j	(phenyl group)	85.6 ± 17.0
8k	H	> 5000
BI-3406		71.8 ± 5.1

[a]IC$_{50}$ values are shown as the mean ± standard deviation (SD) from three replicate experiments.

reported the first-in-class SOS1 PROTAC **2** that potently induced SOS1 degradation and inhibited the growth of multiple human cancer cells harboring various KRAS mutations.[17]

To develop small molecule inhibitors that disrupt the interaction of KRAS-SOS1, Hillig et al. first reported a series of aminoquinazoline compounds as KRAS-SOS1 interaction inhibitors. The representative compound BAY-293 (**3**) with a good SOS1 binding affinity showed synergistic antiproliferative activity when used in combination with the KRAS G12C inhibitor.[18] The other aminoquinazoline compound BI-3406 (**4**), independently discovered by the researchers from Boehringer Ingelheim, showed an improved activity compared with BAY-293.[3] Its close analogue BI 1701963 is currently under phase I clinical trials for treating KRAS-mutated solid tumors. The preliminary result suggested that BI 1701963 was well tolerated, and stable disease control was achieved in 7 of 31 treated patients when administered alone (NCT04111458).[19] Another compound MRTX0902 (**5**) developed by Mirati Therapeutics with a phthalazine core also reached the clinical trial stage recently.[20] Similar works have also been reported by Revolution Medicine and He et al. for the SOS1 inhibitor tool RMC-0331 (**6**) carrying a pyrrolo[3,4-*d*]pyrimidin scaffold[21] and the tetracyclic quinazoline SOS1 inhibitors (e.g., **7**) with favorable druglike properties, respectively.[22]

Our group's research interests focus on the discovery of small molecule probes targeting the KRAS-MAPK pathway. Using the scaffold hopping strategy, we also designed a class of SOS1 inhibitors on the basis of pyrido[2,3-*d*]pyrimidin-7-one, which is a privileged scaffold in drug development.[23] While we were doing this project, some related patents have been filed.[24−26] Herein, we report our efforts in the design, synthesis, and biological evaluation of the new SOS1 inhibitors.

Synthesis of the pyrido[2,3-*d*]pyrimidin-7-ones **8** is described in Scheme 1. We started from the commercially available 4,6-dichloropyrimidine-5-carbaldehydes **9** by reacting them with various amines **10** under basic conditions to obtain intermediates **11**. Compounds **11** were then reacted with methyl glycinate hydrochloride and 4-methoxybenzaldehyde in the presence of Et$_3$N in MeOH. The resulting mixture was heated in 70% AcOH to yield the key intermediates **12**.[27] Compound **8a** was obtained by first reacting the intermediate **12a** with (*R*)-1-(3-nitro-5-(trifluoromethyl)phenyl) ethan-1-amine (**14a**) via the nucleophilic aromatic substitution, followed by the Pd/C-catalyzed nitro reduction reaction. For compounds **8b** and **8k**, the amine group of **12a** was acetylated or deaminated to afford the intermediates **18** and **19**, respectively. The two intermediates were converted to the desired products **8b** and **8k** in the same manner of compound **8a**. For the rest of the compounds, the intermediates **12** were first converted to compounds **13** via the Sandmeyer reaction. After reaction with amines **14**, the resulting compounds **15** underwent Buchwald−Hartwig coupling reactions (with the amines **16**) or Suzuki coupling reactions (with the borate esters **17**) to form the desired products **8**. In some cases, an additional reduction of the nitro group and/or double bond was also required. Compound **8e** was obtained by removing the Boc-protected group from compound **20**, and **8g** was prepared by coupling 1-methylpiperidine-4-carboxylic acid with **8e** in the presence of HATU and DIPEA.

Table 2. Structures and Biochemical Activities of 8f and 8l−x with Different R^2, R^3, and R^4

Compound	R^2	R^3	R^4	SOS1 HTRF IC$_{50}$ (nM)[a]	Compound	R^2	R^3	R^4	SOS1 HTRF IC$_{50}$ (nM)[a]
8f	H$_2$N—/CF$_3$	H	Me	87.8 ± 9.2	8r	CF$_3$	H	Me	138 ± 25
8l	H$_2$N—/CF$_3$	H	Me	690 ± 258	8s	CN	H	Me	226 ± 50
8m	H$_2$N	H	Me	801 ± 171	8t	CF$_3$	H	Me	>5000
8n	CF$_3$	H	Me	134 ± 20	8u	H$_2$N—/CF$_3$	Me	Me	95.4 ± 9.8
8o	O—CF$_3$	H	Me	869 ± 278	8v	H$_2$N—/CF$_3$	H	H	67.4 ± 3.5
8p	F F OH	H	Me	73.4 ± 12.6	8w	H$_2$N—/CF$_3$	H	Et	90.8 ± 19.0
8q	F F OH / F	H	Me	92.8 ± 19.4	8x	H$_2$N—/CF$_3$	H	n-Pr	77.5 ± 9.3

[a]IC$_{50}$ values are shown as the mean ± SD from three replicate experiments.

Inspired by BI-3406, we first kept the compounds with (R)-3-(1-aminoethyl)-5-(trifluoromethyl) aniline at the C4-position and modified the R^1 group (Table 1). The ability of the designed compounds to inhibit the SOS1-mediated KRAS^{G12D} activation was evaluated by a homogeneous time-resolved fluorescence (HTRF) assay.[18] The starting compound 8a with an amine group at the R^1 position did not show any measurable activity against SOS1 (IC$_{50}$ > 5000 nM). Its acetyl derivative 8b remained inactive. Interestingly, the introduction of a cyclopentyl group gained an IC$_{50}$ value of 463 nM (8c),

while the slightly larger cyclohexyl ring (8d) caused the loss of activity again. When the R^1 substituent was replaced with a piperidine, the resulting compound 8e showed a substantial increase of the SOS1 inhibition activity compared with 8c, with an IC$_{50}$ value of 94.0 nM. Acetylation of the piperidine did not impair the inhibition activity of 8e to any significant extent, as reflected by compounds 8f and 8g with IC$_{50}$ values of 87.8 and 171 nM, respectively. The replacement of the acetyl piperidine of 8f with the acetyl piperazine resulted in compound 8h, which was equipotent to the lead compound BI-3406 (4). The
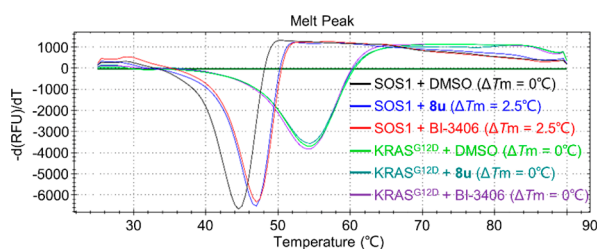
Figure 2. **8u** stabilized SOS1 but not KRAS^{G12D} in a thermal shift assay.
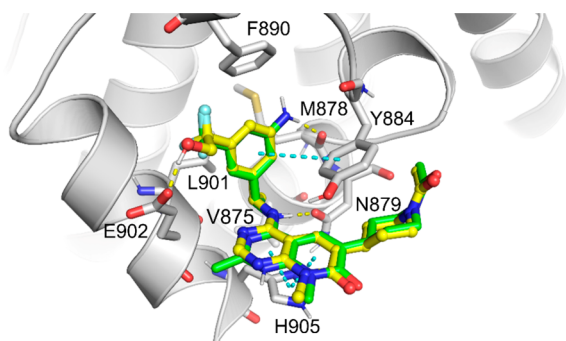

Figure 3. Modeled binding poses of **8p** (yellow) and **8u** (green) in SOS1 (gray, modeled with PDB: 6SCM).

Table 3. Proliferation Inhibitory Activities of Selected SOS1 Inhibitors in MIA PaCa-2 Cells

compound	IC$_{50}$ (nM)^a
8e	160 ± 37
8f	190 ± 20
8h	92.0 ± 29.9
8i	74.8 ± 19.3
8j	429 ± 156
8p	156 ± 37
8q	459 ± 86
8u	27.7 ± 5.7
8v	90.1 ± 22.3
8w	340 ± 57
8x	317 ± 87
BI-3406	30.0 ± 4.6

^{a}IC$_{50}$ values are shown as the mean ± SD from four replicate experiments.

aromatic substituents of the pyridyl (**8i**) and phenyl (**8j**) groups also maintained the good inhibition activity of these compounds. However, the absence of the R^1 substituent caused a complete loss of the SOS1 inhibition activity of compound **8k**. These data together emphasize the importance of the existence of the R^1 substituent with a good tolerance of its bulkiness and hydrophobicity.

By fixing the R^1 substituent as the 1-acetylpiperidin-4-yl group, we conducted the next round of SAR study at the C4-position (Table 2). Removal of the chiral methyl group of **8f** dramatically decreased the activity of the resulting compound **8l**, thereby confirming the essential role of the chiral center observed in other studies.[18] Removal of the CF$_3$ substituent of the phenyl ring decreased the activity by about 9-fold (**8m**), while removal of the amino group (**8n**) only caused less than 2-fold loss of the activity with an IC$_{50}$ value of 134 nM. We then made more modifications on the phenyl ring without an

amino group. Replacement of 3-CF$_3$ (**8n**) with 3-OCF$_3$ (**8o**) led to an ∼7-fold decreased potency, while replacement of this trifluoromethyl with a 1,1-difluoro-2-hydroxyethyl substituent (**8p**) slightly improved the activity to 73.4 nM. The incorporation of an additional small substituent such as F (**8q** vs **8p**) or Me (**8r** vs **8n**) on the phenyl ring did not remarkably change the activity. Replacement of CF$_3$ of **8r** with the CN group (**8s**) slightly reduced the activity to 226 nM. The truncation of the methylene group (**8t**) caused the complete loss of the inhibition activity of compound **8n** (IC$_{50}$ > 5000 nM).

The effect of the substituents at the R^3 and R^4 positions on the SOS1 inhibition activity was also studied, as reflected by compounds **8u**−**x**. We found that adding a methyl substituent at R^3 was well tolerated for SOS1 binding, and the resulting compound **8u** obtained an IC$_{50}$ of 95.4 nM. A similar result was also observed for the substitution at the R^4 position, with proton (**8v**), Et (**8w**), and n-Pr (**8x**) being almost equipotent compared with the Me substituent (**8f**) (Table 2).

To demonstrate that the newly obtained compound **8** directly binds to SOS1 rather than KRAS^{G12D}, which was used in the HTRF assays, we performed a thermal shift assay (TSA) by using **8u** as a model compound. The results showed that **8u** stabilized the SOS1 protein with a ΔT$_m$ of 2.5 °C, but not KRAS^{G12D}, in the same way as did BI-3406 (Figure 2).

A molecular simulation study also provided a similar binding mode of the newly synthesized compounds **8p** and **8u** when compared with BI-3406.[3] As illustrated in Figure 3, the pyrido[2,3-d]pyrimidin-7-one core orientates in a parallel position with His905 and forms the π−π stacking interaction. A key hydrogen bond is formed between the NH adjacent to the pyrido[2,3-d]pyrimidin-7-one core and the Asn879 residue. The C4-substituted phenyl moiety occupies the hydrophobic pocket comprised of Phe890, Leu901, and Tyr884. Additional hydrogen bonds formed by the NH$_2$ of **8u** with Met878 and the OH of **8p** with Glu902 are also observed. The importance of the chiral methyl group could be due to its control of the favorable binding orientation and the tight occupancy of a small hydrophobic binding pocket formed by Leu901 and Val875. The acetylpiperidinyl group points outward of the binding pocket and, thus, results in minimal changes to the SOS1 inhibition activities after extensive structural modification. The small hydrophobic substitutions at the N8-position were also well tolerated because this position is similarly pointing toward out of the binding pocket.

To evaluate the antiproliferative effects of the SOS1 inhibitors, we first tested the growth-inhibitory activity of selected compounds against MIA PaCa-2 tumor cells carrying G12C-mutated KRAS using the 3-D growth assays (Table 3 and Figure S1). As observed with the reported SOS1 inhibitor BI-3406, the tested compounds **8** only achieved the maximal cell growth inhibition around 60% at high concentrations and obtained the IC$_{50}$ values between 27.7 and 459 nM as half-maximum inhibitory concentration. The most potent compound **8u** displayed comparable activity with BI-3406. Since the new SOS1 inhibitors were developed on the basis of the pyrido[2,3-d]pyrimidin-7-one scaffold that has been widely used in the design of kinase inhibitors, in order to avoid any potential off-target effect of kinase inhibition, we tested **8u** against a small panel of kinases, including FGFR1,[28] EGFR,[29] HER2, PDGFRα,[30] CDK2,[31] and CDK4,[23] with inhibitors carrying the same scaffold. To our satisfaction, **8u** displayed an excellent selectivity over the tested kinases (IC$_{50}$ values > 10
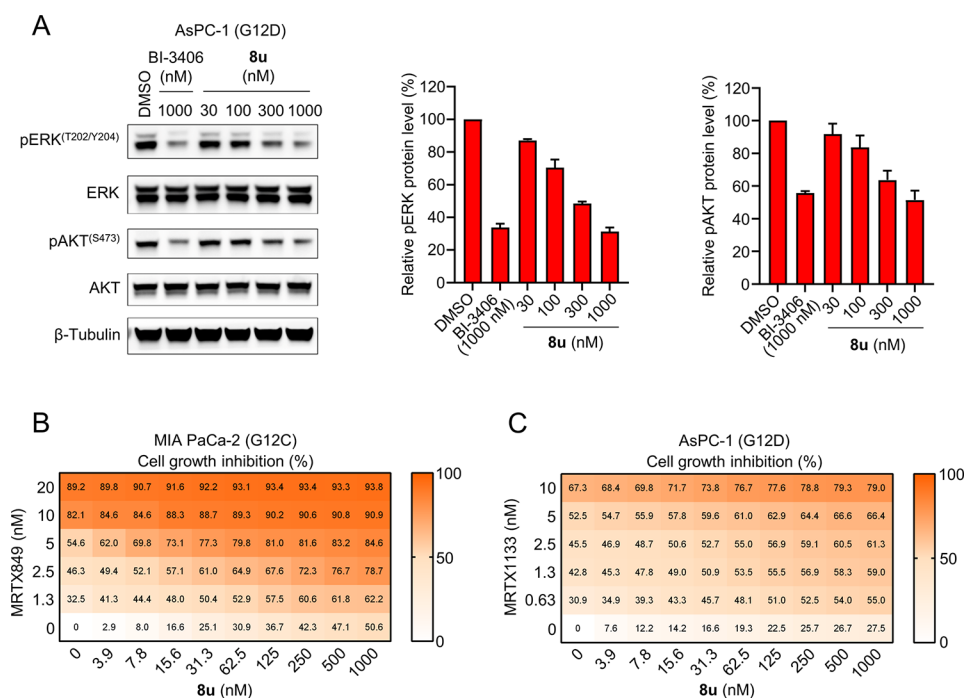
Figure 4. (A) Representative immunoblots of pERK and pAKT in AsPC-1 (G12D) cells that were treated with the indicated concentration of **8u** for 2 h. pERK and pAKT levels were quantified (right panel). Quantified data represent the mean $\pm$ SD from two independent biological replicates. (B and C) 3-D antiproliferative activity of **8u** in combination with the $KRAS^{G12C}$ inhibitor MRTX849 in MIA PaCa-2 cells (B) and $KRAS^{G12D}$ inhibitor MRTX1133 in AsPC-1 cells (C). ($n = 2$; values calculated from the mean).

μM, Figure S2). Compound **8u** was further assessed in a panel of tumor cells with various KRAS statuses (Figure S3). It showed good activities against the cell lines with G12C, G12D, or G12V-mutated KRAS, and the IC_{50} values were 3.56, 6.28, 10.7, and 16.9 nM in NCI-H358 ($KRAS^{G12C}$), AsPC-1 ($KRAS^{G12D}$), PANC-1 ($KRAS^{G12D}$), and SW620 ($KRAS^{G12V}$) cell lines, respectively. Compound **8u** obtained moderate activity against A549 cells harboring the KRAS G12S mutation (IC_{50} = 115 nM). In contrast, it did not inhibit the growth of A375 cells that carry wild-type KRAS and is independent of KRAS for viability.[3]

Furthermore, compound **8u** dose-dependently inhibited the phosphorylation of ERK and AKT—the two downstream signals of KRAS—in the AsPC-1 cells with the $KRAS^{G12D}$ mutant (Figure 4A). It also reduced the levels of phosphorylated ERK and AKT in MIA PaCa-2 tumor cells with the $KRAS^{G12C}$ mutant in the same but relatively weak manner (Figure S4).

Previous reports have shown enhanced tumor growth inhibition of SOS1 inhibitors when administered together with the MEK or $KRAS^{G12C}$ inhibitors.[3,20] The same synergistic effect was also observed with our new pyrido[2,3-d]pyrimidin-7-one SOS1 inhibitors (Figure 4B,C). The tumor cell growth inhibition was enhanced in an obviously synergistic manner when treating the MIA PaCa-2 and AsPC-1 cell lines with compound **8u** in combination with MRTX849 ($KRAS^{G12C}$ inhibitor)[10] or MRTX1133 ($KRAS^{G12D}$ inhibitor).[7] These results further demonstrated the potential combination use of the SOS1 and mutant-selective KRAS inhibitors.

In summary, we designed and synthesized a new series of SOS1 inhibitors with the pyrido[2,3-d]pyrimidin-7-one scaffold. Systematic structural modification and activity evaluation led to the identification of **8u** with equipotent activity when compared with the parental compound BI-3406. Compound

8u showed an excellent selectivity against 6 kinases with inhibitors carrying the same scaffold and displayed good tumor cell growth-inhibitory activities in a panel of G12-mutated KRAS-driven cancer cells. It also induced dose-dependent inhibition of the phosphorylation of the downstream signals ERK and AKT. More strikingly, we found that our SOS1 inhibitor was able to work synergistically with KRAS G12C and G12D inhibitors and obtained enhanced cytotoxicity. These data together show the good promise of compound **8u** as a pan-KRAS inhibitor.

◼ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsmedchemlett.2c00490.

Details of biological assays and chemical synthesis (PDF)

◼ AUTHOR INFORMATION

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS

KRAS, Kirsten rat sarcoma virus; GEFs, guanine nucleotide exchange factors; GAPs, GTPase-activating proteins; SOS1, Son of Sevenless 1; ERK, extracellular signal-regulated kinase; AKT, protein kinase B; PROTAC, proteolysis-targeting chimera; MAPK, mitogen-activated protein kinase; HATU, hexafluorophosphate azabenzotriazole tetramethyl uronium; DIPEA, diisopropylethylamine; DCM, dichloromethane; HTRF, homogeneous time-resolved fluorescence; TSA, thermal shift assay

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