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AcGlcAs: A Novel P53-Targeting Arsenical with Potent Cellular Uptake and Cancer Cell Selectivity

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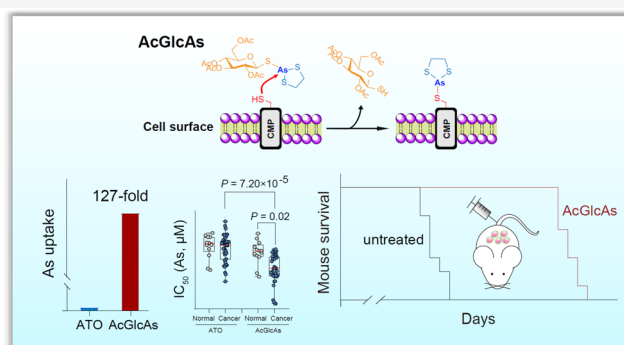


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ABSTRACT: Arsenic trioxide (ATO) targets PML/RAR α and leads to miraculous success in treating acute promyelocytic leukemia. Notably, ATO also targets p53, the most frequently mutated protein in cancers, through a similar binding mechanism. However, p53-targeting ATO trials are challenging due to the poor cellular uptake and cancer selectivity of ATO. Here, we analyzed the structure–activity relationship of arsenicals and rationally developed a novel arsenical (designated AcGlcAs) by conjugating arsenic to sulfur atoms and tetraacetyl- β -D-thioglucose. AcGlcAs exhibited remarkable cellular uptake through a thiol-mediated pathway (maximally 127-fold higher than ATO), thereby potentially targeting PML/RAR α and mutant p53. Among the 55 tested cell lines, AcGlcAs preferentially killed cancer lines rather than normal lines. In preclinical studies, AcGlcAs significantly extended the survival of mice bearing a xenograft tumor with p53 mutation while showing high plasma stability and oral bioavailability. Thus, AcGlcAs is a potential clinical candidate for precisely treating numerous p53-mutated cancers.



INTRODUCTION

Arsenicals have been used therapeutically for more than 2400 years.¹ Arsenic trioxide (ATO), in combination with all-trans retinoic acid, produced over 90% 5 year disease-free survival in acute promyelocytic leukemia (APL) patients, making APL the only malignancy definitively cured by targeted therapies to date.^{2,3} Mechanistically, ATO induced degradation of the APL-specific oncoprotein PML/RAR α by the covalent binding of released arsenic (As) atom to the cysteine-triad pocket of PML moiety.⁴

The tumor suppressor p53 is inactivated by mutations in approximately half of all cancers.^{5–7} Developing a drug that can rescue the tumor-suppressive function of mutant p53 has long been considered the “Holy Grail” of targeted anticancer therapy.^{8,9} We recently reported that ATO could rescue structural p53 mutants, with potencies one or more orders of magnitude higher than the large number of other reported p53-rescue compounds.¹⁰ Interestingly, ATO targets p53 by the As atom’s covalent occupation of a cryptic cysteine-triad pocket of p53—a mechanism similar to its targeting of PML/RAR α .¹⁰ Thus, arsenicals have immense potential for treating the large population of patients with p53-mutated cancers, with more than 8 million new cases per year.

ATO was repurposed to treat non-APL cancers in over 100 trials registered at [ClinicalTrials.gov](https://clinicaltrials.gov) in the past two decades, including a series of recent p53-targeting trials inspired by our study (e.g., NCT04869475, NCT04489706, and NCT04695223). The major challenge for these trials lies in the low effective concentrations of arsenic in cancer cells due to poor cellular uptake. No ATO-specific cellular uptake pathway has been reported to date.¹¹ Instead, ATO enters cells by hijacking some nonspecific transporters,¹¹ such as the well-established aquaglyceroporin channel AQP9.^{12,13} The miraculous efficacy of ATO in treating APL leukemia is partially attributed to the particularly high expression of AQP9 in APL compared to non-APL leukemias.¹⁴ In addition, arsenic is rapidly eliminated from the human body.^{15,16} Such poor pharmacokinetics (PK) also contributes to the low accumulation of arsenic in cancer cells *in vivo*. The second challenge for

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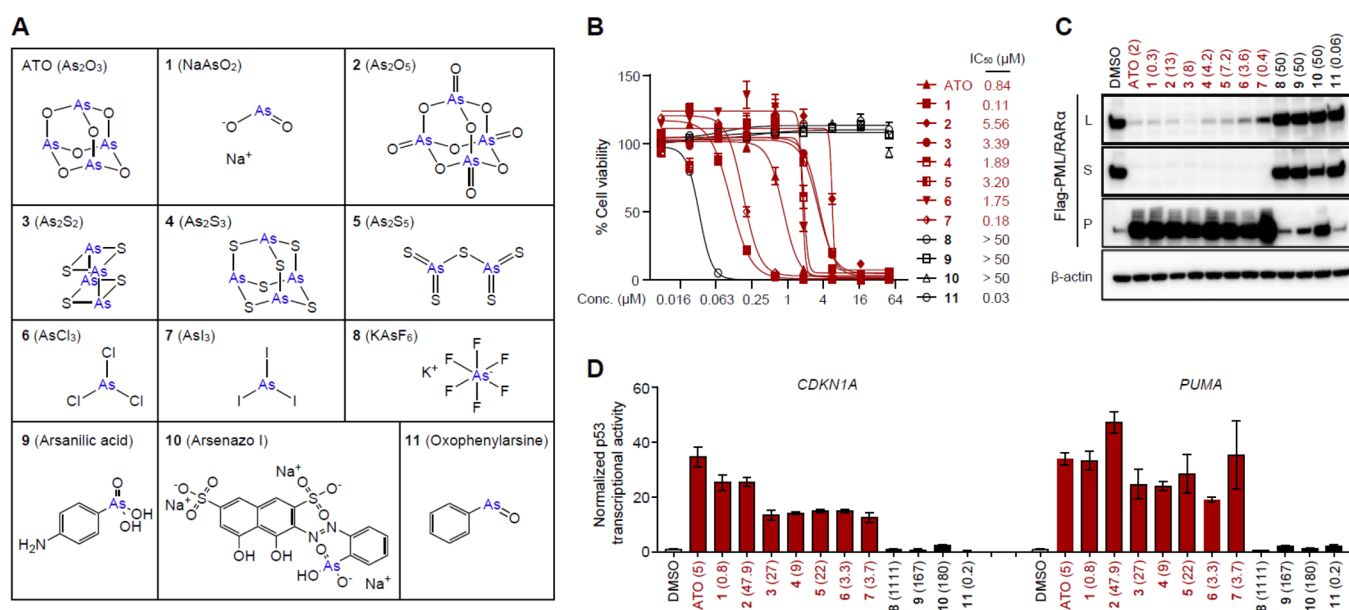


Figure 1. SAR analysis of 12 arsenicals. (A) Chemical structures of the tested arsenicals. (B) Viability of NB4 cells according to the CCK-8 assay after treatment with the indicated arsenical for 48 h. (C) Effect of arsenicals on degradation of PML/RAR α after 24 h of treatment in 293T cells transfected with Flag-PML/RAR α , as indicated by western blotting using Flag antibody. Concentrations are indicated in parentheses (μ M). L, whole cell lysates; S, supernatants; P, pellets. (D) Effect of arsenicals on p53-V272M transcriptional activity on the indicated p53 target gene promoters after treatment for 24 h in H1299 cells, as indicated by a luciferase reporter assay. Concentrations are indicated in parentheses (μ M).

ATO repurposing trials lies in a lack of cancer cell selectivity. In fact, since the effective dosages of ATO in the treatment of non-APL cancers are much higher (approximately 12–40-fold) than that used in treating APL,¹⁷ such high doses were usually accompanied by severe systemic toxicity.^{18,19}

A variety of approaches, such as encapsulation with liposomes^{20,21} or polymeric micelles,^{22,23} were investigated with the aim to improve the cellular uptake and cancer cell selectivity of arsenicals in the past decade. Recently, a promising ferritin-based As nanocarrier (As@Fn) was reported.²⁴ Benefiting from the interaction between ferritin and CD71 that is overexpressed in leukemia cells, As@Fn displayed more efficient cellular uptake than ATO and therefore achieved high efficacy in treating a variety of leukemia mouse models.²⁴ However, the potential of As@Fn in treating solid tumors without established CD71 overexpression remains unclear. Another example is the recently developed biocompatible PEGylated arsenene nanodot (AsNDs@PEG) that preferentially inhibits cancer cells due to a cancer cell-specific valence change of arsenic.²⁵ However, these exciting macromolecular and nanomaterial-based approaches are challenged by the relatively low stability of arsenic complexes during storage and unexpected drug release during circulation in the human body.²⁶ An alternative is to chemically modify As with small-molecule groups, and one such arsenical, darinaparsin, showed efficacy in phase I studies in relapsed/refractory peripheral T-cell lymphoma.²⁷ However, darinaparsin could not induce PML/RAR α degradation in APL,²⁸ raising concerns about its potential for the broad-spectrum treatment of cancers with p53 mutations. It is therefore necessary to reveal the structure–activity relationship (SAR) of arsenicals in cells, through which arsenicals that overcome the two drawbacks of ATO can be rationally designed without losing the ability to rescue mutant p53.

Cancer cells have more abundant surface thiols than the paired nontransformed cell types.^{29–32} Thus, the thiol-mediated uptake, which makes use of dynamic covalent interactions with

cell surface thiols to deliver cargos into cells with high efficiency,^{33,34} is a promising approach to efficiently and selectively delivering As into cancer cells. For example, antitumor immunity-stimulating agents, TLR7 agonists, were recently conjugated with pyridyl disulfide (PDS).³⁵ The resultant compounds underwent efficient disulfide exchange with tumor-surface thiols and consequently induced robust antitumor response. Here, we analyzed the SAR of arsenicals and rationally developed a novel arsenical named AcGlcAs that delivers As into cells through thiol-mediated uptake. AcGlcAs exhibited efficient cellular uptake as well as high PML/RAR α - and p53-targeting activities, thereby potently and selectively killing cancer cells. The further *in vivo* analyses of anticancer activity and PK in mice suggest that AcGlcAs is a potential clinical candidate for the treatment of p53-mutated cancers.

RESULTS AND DISCUSSION

SAR of 12 Arsenicals. To reveal the prerequisites for an arsenical to effectively target PML/RAR α and mutant p53, we collected 12 structurally diverse arsenicals, including ATO (Figure 1A). In the APL cell line NB4-expressing PML/RAR α , eight arsenicals (1–7 and 11) exhibited significant cytotoxicity (IC₅₀ < 10 μ M). At concentrations close to the IC₅₀ in NB4, all eight arsenicals, except for 11, efficiently degraded PML/RAR α . This was reflected in changes of solubility (transferring of PML/RAR α from the soluble supernatant (S) of whole cell lysates (L) to insoluble pellet (P)) (Figure 1C and Figure S1). Transcriptional activity is central to the tumor-suppressive function of p53. Among the 12 arsenicals, 1–7 efficiently restored the transcriptional activity of p53-V272M (an established structural p53 mutant that can be rescued by ATO¹⁰) on the representative p53 target genes *CDKN1A* and *PUMA*³⁶ (Figure 1D).

These results and the chemical properties of the tested arsenicals are summarized in Figure S2. Based on this overview, we concluded that the arsenicals with stable As–C or relatively

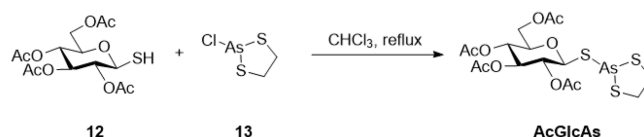
stable As–F bonds, as exemplified by arsenicals **8–11**, failed to efficiently target PML/RAR α and mutant p53. These arsenicals were most likely unable to release the As atom to interact with the thiol groups of the cysteine-triad pocket. This SAR further explains the previously reported elusive findings in which arsenicals that contain As–C bonds, including darinaparsin²⁸ (Figure S3), melarsoprol,³⁷ and mono- and dimethylated metabolites of As,³⁸ failed to induce the degradation of PML/RAR α . Thus, to retain the PML/RAR α - and mutant p53-targeting activity, the As atom should not be embedded in a structure with stable As–C bonds.

Rational Design of the Novel Arsenical AcGlcAs. A variety of thiol-reactive biomolecules, including small molecules, macromolecules, and drug carriers, were reported to exhibit potent cellular association and internalization.^{30,39} A well-studied example is the rheumatoid arthritis drug auranofin (Figure 2A, left panel), which contains a phosphine-coordinated

the form of a 5-membered ring to ensure structural stability as reported previously.⁴¹ At the same time, such coordination ensures that the molecule retains the capacity to target PML/RAR α and p53 mutants as revealed by SAR. Afterward, we conjugated the As atom with TATG via the third As–S bond, envisaging a cellular uptake mechanism analogous to that of auranofin.⁴⁰ Since a set of thiol-containing cell membrane proteins (CMPs), including the extensively reported transmembrane protein TFRC that is highly expressed in cancers, were proposed as membrane targets of thiol-mediated uptake,^{31,34} we proposed a mechanism of cell association and internalization for the designed arsenical as shown in Figure 2B.

The generated arsenical, designated as AcGlcAs, was synthesized as shown in Scheme 1. Briefly, a solution of

Scheme 1. Synthetic Route of AcGlcAs^a



^aReagents and conditions: tetraacetyl- β -D-thioglucose (**12**) and 2-chloro-1,3-dithia-2-arsacyclopentane (**13**) (1:1), CHCl₃, reflux, 6 h, 60%.

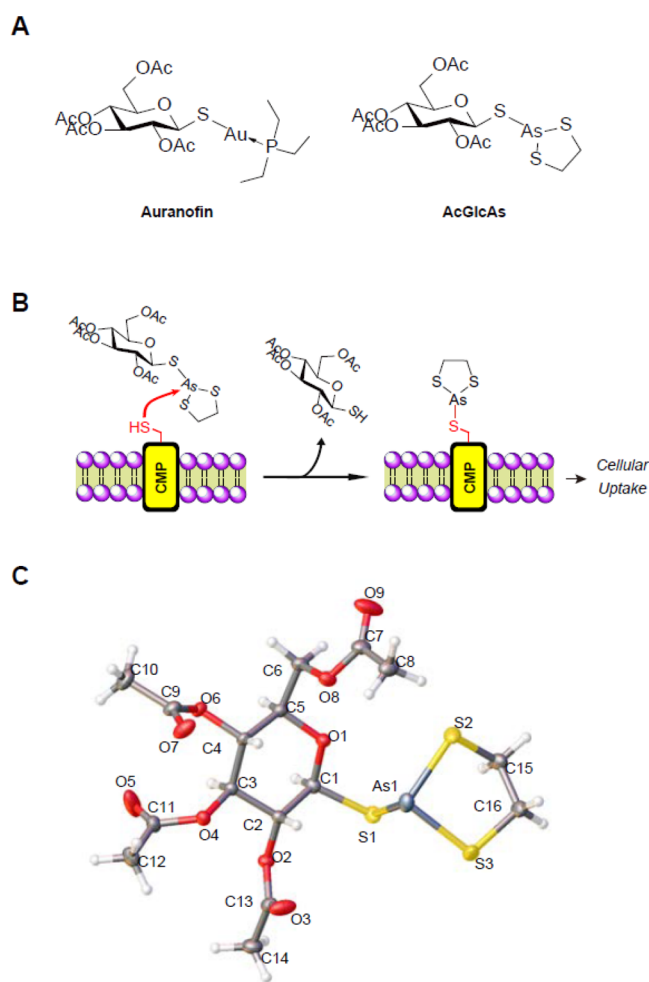


Figure 2. Rational design of the novel arsenical AcGlcAs. (A) Chemical structures of auranofin and newly designed AcGlcAs. (B) Proposed cellular uptake mechanism of AcGlcAs. (C) Determined X-ray crystal structure of AcGlcAs.

gold atom (Au) linked to the tetraacetyl- β -D-thioglucose (TATG) moiety.⁴⁰ Cell surface thiols can compete with TATG for substitution of the Au–S coordinate bond, leading to rapid cell association and uptake of the gold atom.⁴⁰ Thus, we rationally designed a novel arsenical as follows (Figure 2A, right panel). The ethanedithiol moiety was chosen to coordinate As in

tetraacetyl- β -D-thioglucose⁴² in CHCl₃ was added to 2-chloro-1,3-dithia-2-arsacyclopentane⁴³ in CHCl₃. The mixture was heated under reflux. The product was obtained in 55% yield after a work-up procedure and with 99% purity determined by HPLC. The successful synthesis of AcGlcAs was confirmed by proton nuclear magnetic resonance (¹H NMR, Figure S3) and carbon nuclear magnetic resonance (¹³C NMR, Figure S4). High-resolution electrospray ionization mass spectrometry (ESI-HRMS) gave an *m/z* value of 552.9616, which could be attributed to C₁₆H₂₃AsO₉S₃Na (theoretically calculated mass: 552.9618). Elemental analysis gave the anal. (C₁₆H₂₃AsO₉S₃) C, H, and N. Moreover, the chemical structure of AcGlcAs was further confirmed by X-ray crystallography (CCDC 2122393). The X-ray crystal structure clearly showed that the As moiety was attached to the 1-S-D-glucose position (Figure 2C and Table S1).

Cellular Uptake of AcGlcAs through the Thiol-Mediated Pathway. We next explored the cellular uptake of AcGlcAs (Figure 3A), subcellular localization, and its dependency on surface thiols by inductively coupled plasma mass spectrometry (ICP-MS). After treatment with ATO or AcGlcAs at the equimolar As concentration (10 μ M) for 10 min, the accumulation of As in the AcGlcAs-treated cells was 19- to 127-fold higher than that in the ATO-treated cells (Figure 3A). Such efficiency is superior to the promising As@Fn, which achieved an approximately 4.5-fold increase in cellular As uptake compared to ATO.²⁴ The subcellular localization of As in HepG2 was determined by ICP-MS. After treatment with 10 μ M AcGlcAs for 1 h, As could be detected in not only the cytoplasm but also the nucleus (Figure 3B). This makes it possible for As to bind nuclear proteins such as PML and mutant p53. Moreover, preblocking cellular thiols with 100 μ M *N*-ethylmaleimide (NEM), a sulfhydryl alkylating reagent, which was reported to effectively impair the thiol-mediated uptake of auranofin,⁴⁰ resulted in an approximately 90% reduction of cellular As content compared to the untreated group (Figure 3C). As expected, the effect of AcGlcAs in transcriptionally reactivating

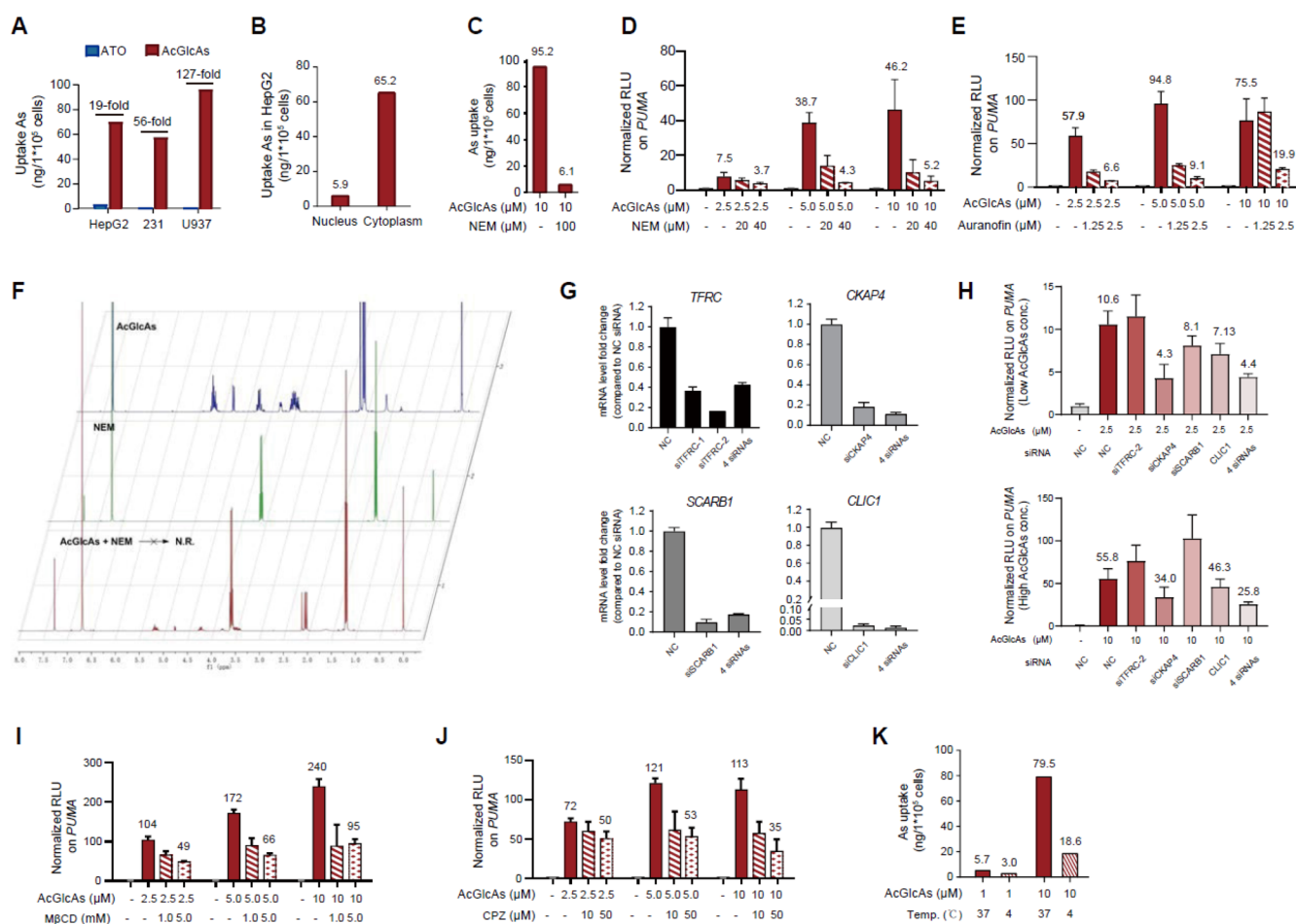


Figure 3. Cellular uptake of AcGlcAs through the thiol-mediated pathway. (A) As contents in HepG2, MDA-MB-231, and U937 cells after treatment with AcGlcAs or ATO at equimolar As concentration (10 μM) for 10 min, as determined by ICP-MS. (B) As content in the nucleus and cytoplasm of HepG2 cells after 1 h of treatment of 10 μM AcGlcAs, as determined by ICP-MS. (C) As content in HepG2 cells after pretreatment with 0 or 100 μM NEM for 30 min, followed by treatment with 10 μM AcGlcAs for 10 min, as determined by ICP-MS. (D) Effect of NEM pretreatment on the potency of AcGlcAs in reactivating p53-V272M transcriptional activity on the PUMA promoter in H1299 cells. Cells were preincubated with or without NEM for 30 min, followed by treatment with 10 μM AcGlcAs for 10 min and incubation with fresh medium for another 24 h. (E) The effect of Auranofin pretreatment on the potency of AcGlcAs in reactivating p53-V272M transcriptional activity on the PUMA promoter was determined in H1299 cells. Cells were preincubated with or without NEM for 10 min, followed by treatment with 10 μM AcGlcAs for 10 min and incubation with fresh medium for another 24 h. (F) The mixture of AcGlcAs (10 μM) and NEM (100 μM) in PBS was incubated for 1 h at RT. After that, the mixture was recovered by extraction and determined by ¹H NMR. The ¹H NMR spectra of standards and recovered drugs are shown. There was no sign of new compounds according to the comparison of parallel ¹H NMR spectra between standards and the recovered drugs. No reaction, N.R. (G) RT-qPCR analysis of mRNA levels of the indicated genes in H1299 cells after siRNA transfection for 3 days. (H) Effect of siRNA knocking down on the potency of AcGlcAs in reactivating p53-V272M transcriptional activity on the PUMA promoter in H1299 cells, as indicated by the luciferase reporter assay. (I, J) Effect of endocytosis inhibitor MβCD (I) or CPZ (J) on the potency of AcGlcAs in reactivating p53-V272M transcriptional activity on the PUMA promoter in H1299 cells, as indicated by the luciferase reporter assay. The treatment method was the same as in panel (D). (K) As contents in HepG2 cells upon incubation with 1 or 10 μM AcGlcAs at 37 or 4 °C for 5 min, as determined by ICP-MS.

p53-V272M on the PUMA promoter was greatly reduced by pretreatment with both 20 and 40 μM NEM (Figure 3D). Interestingly, 1.25–2.5 μM Auranofin treatment effectively inhibited AcGlcAs-mediated mutant p53 transcriptional reactivation (Figure 3E). Since Auranofin is known to enter cells through the thiol-mediated pathway,⁴⁰ a likely explanation for this result is that Auranofin competes with AcGlcAs by employing the same thiol-mediated pathway.

To exclude the possibility of direct reaction between AcGlcAs and NEM, which potentially leads to the observed impairment of As uptake and mutant p53 reactivation of AcGlcAs in the presence of NEM (Figure 3C,D), respectively, the reactivity between AcGlcAs and NEM was determined. AcGlcAs (10 μM) was incubated with NEM (100 μM) in PBS for 1 h at room temperature, accompanied by instant TLC tests. According to

the TLC monitoring results, we did not observe any detectable reaction between these two compounds, as supported by the comparison of parallel ¹H NMR spectra between standards and the recovered drugs' mixture after incubation (Figure 3F). This result excludes the possibility of a direct reaction between AcGlcAs and NEM in our assays.

We hypothesized that the observed selectivity of AcGlcAs toward cancer cells was mediated by CMPs, as proposed in Figure 2B. To this, we focused on 4 CMP-encoding genes *TFRC*, *CKAP4*, *SCARB1*, and *CLIC1*, which were annotated as plasma membrane localization in the GO term database and reported to be involved in thiol-mediated uptake.³¹ We knocked down these four genes individually and determined p53 transactivation activity on PUMA by a luciferase reporter assay. We first confirmed that the four genes could be efficiently knocked down

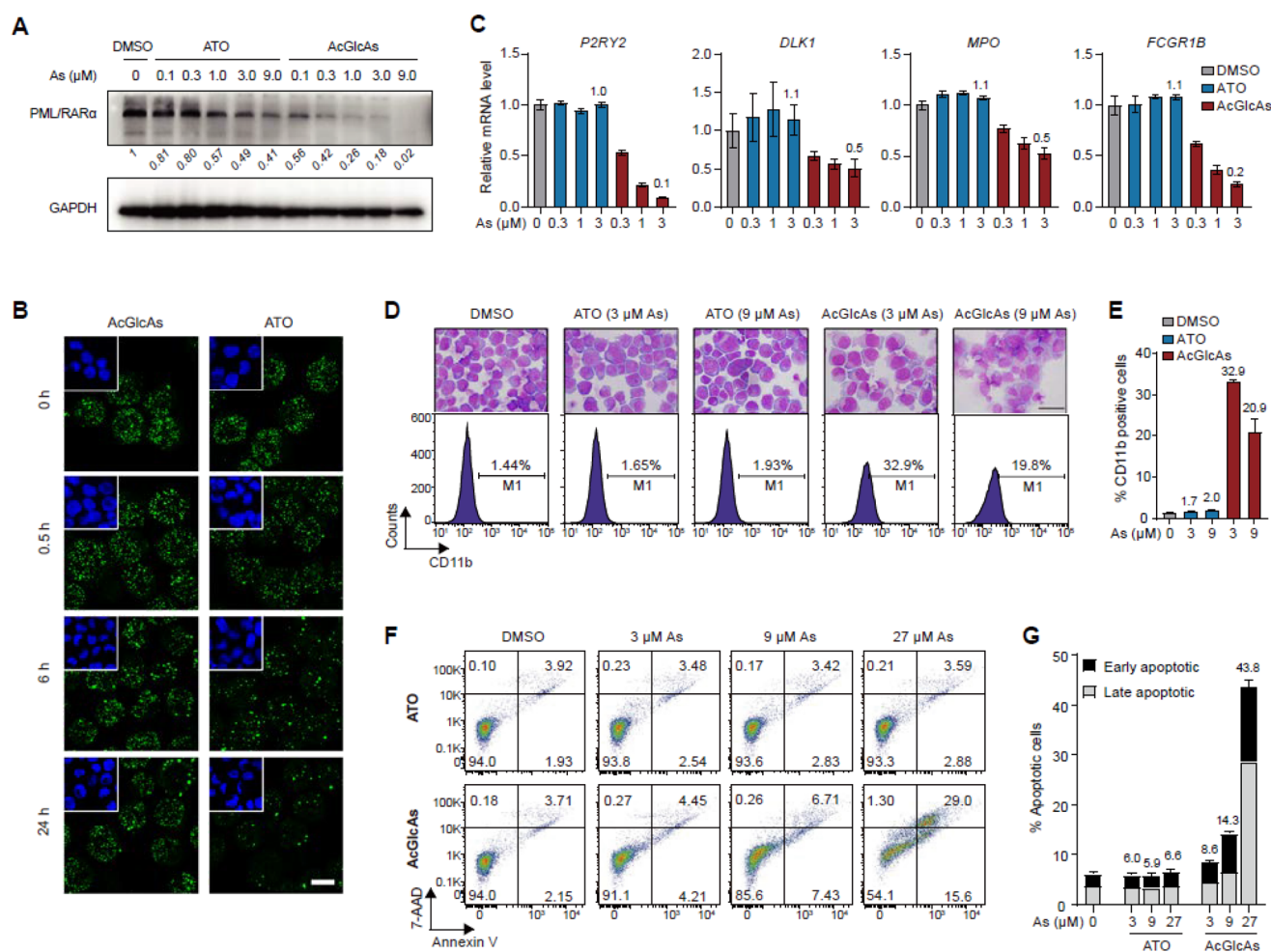


Figure 4. AcGlcAs induced the degradation of the oncoprotein PML/RAR α with high potency. (A) Determination of PML/RAR α levels in NB4 cells by western blot analysis. Cells were treated with a concentration gradient of ATO or AcGlcAs for 1 h and cultured in fresh medium for another 16 h prior to PML/RAR α determination using RAR α antibody. Values are expressed as fold changes of PML/RAR α in the ATO or AcGlcAs group relative to the DMSO group. (B) Immunofluorescence staining of PML/RAR α in NB4 cells. Cells were treated with ATO or AcGlcAs (3 μ M As) for 1 h, followed by culture in fresh medium for the indicated durations and PML/RAR α (green) immunofluorescence staining using PML antibody. DAPI (blue) was used to counter-stain the nuclei. Scale bar, 10 μ m. (C) RT-qPCR analysis of mRNA levels of the indicated downstream genes of PML/RAR α in NB4 cells. Cells were treated with AcGlcAs or ATO for 1 h and cultured in fresh medium for another 6 h. (D, E) Effects of AcGlcAs on the granulocytic differentiation of NB4 cells. Representative profiles are shown in panel (E). Experiments were carried out in triplicate. Cells were treated with ATO or AcGlcAs for 1 h and subsequently cultured in fresh medium for 48 h. The morphology was detected by Wright-Giemsa staining (upper panel), while the expression of the cell surface differentiation marker CD11b was determined by flow cytometry (lower panel). M1 indicates the cell population with high CD11b expression. (F, G) Effects of AcGlcAs on apoptosis of NB4 cells as assessed by flow cytometry using Annexin V-FITC and 7-AAD staining. Representative profiles are shown in panel (F). Cells were pretreated with AcGlcAs or ATO for 1 h, followed by culture with fresh medium for 24 h.

in our experiments (Figure 3G). Among them, knocking down of *CKAP4* and *CLIC1* most efficiently impaired the uptake of AcGlcAs, as suggested by the reduction of AcGlcAs-mediated transcriptional reactivation of mutant p53 on the *PUMA* promoter (Figure 3H). We noticed that simultaneous knocking down of the 4 genes could not completely abrogate the transcriptional reactivation of mutant p53 mediated by AcGlcAs, implying that there might be some additional CMPs involved in AcGlcAs uptake (Figure 3H, the last bars). It was worth noting that 70% of pancreatic ductal adenocarcinomas, lung adenocarcinomas, and other cancer types express high levels of *CKAP4* as compared to adjacent normal tissue.⁴⁴ In addition, *CLIC1* was not expressed in the normal urothelium but highly expressed in dysplastic urothelium, carcinoma in situ, and 94% of the cases with invasive urothelial carcinoma.⁴⁵ Thus, the overexpression of the CMPs that mediate AcGlcAs uptake, such

as *CKAP4* and *CLIC1*, on the cancer cell membrane possibly produces the desired cancer cell selectivity of AcGlcAs.

It was reported that thiol-mediated uptake exploits standard endocytic pathways to deliver cargo-CMP into the cytoplasm.^{34,46} Indeed, AcGlcAs-mediated transcriptional reactivation of mutant p53 was significantly reduced by the two endocytosis inhibitors, methyl- β -cyclodextrin (M β CD, inhibiting caveolae-mediated endocytosis) and chlorpromazine (CPZ, inhibiting clathrin-mediated endocytosis) (Figure 3I,J). This supports the fact that the efficient cellular uptake of AcGlcAs is mediated by cell surface thiols and is at least partially through subsequent endocytosis.

Passive diffusion is commonly involved in cell absorption for a variety of small molecules.⁴⁷ We next determined whether passive diffusion—a process that does not consume energy—is a contributing factor to the cellular uptake of AcGlcAs. A well-

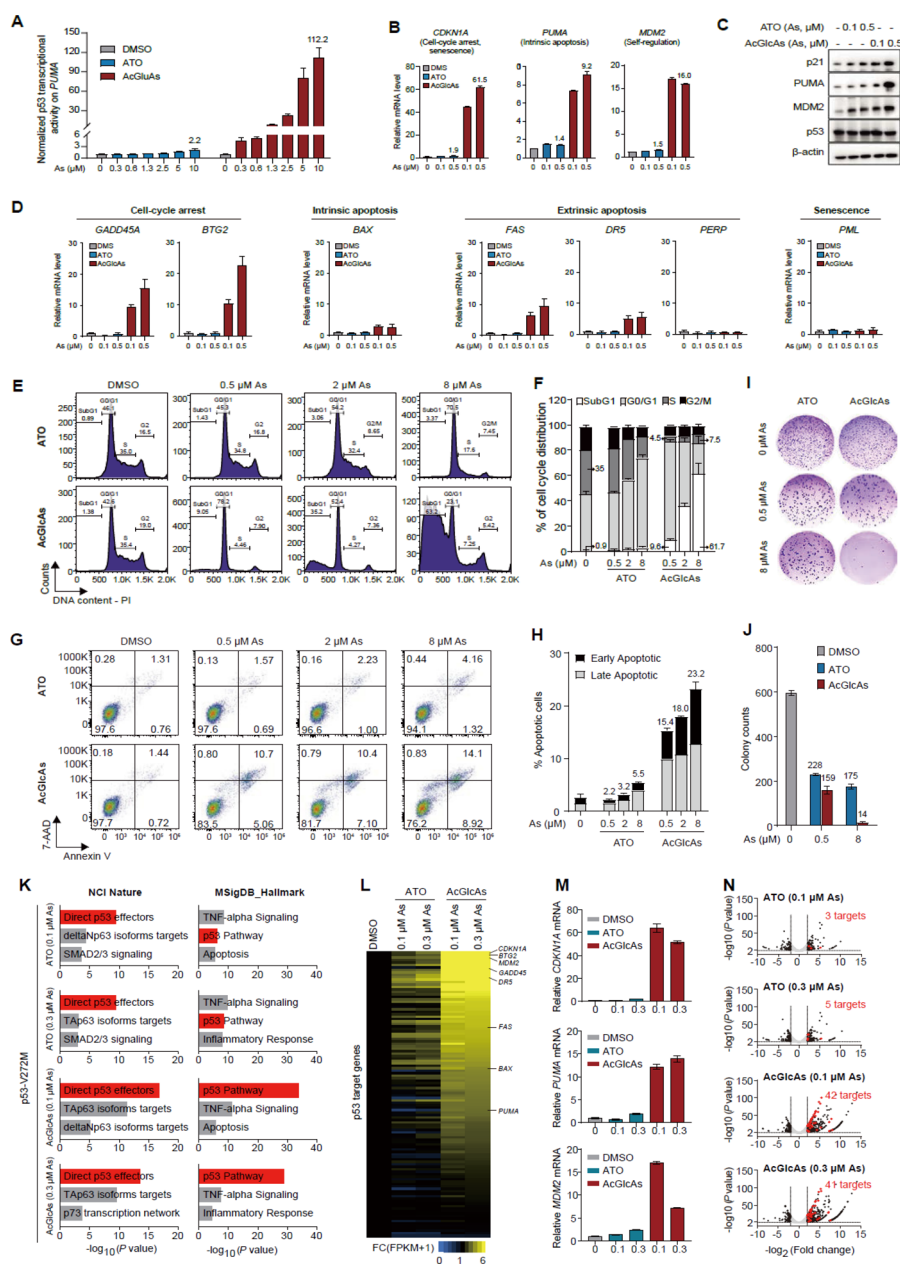


Figure 5. AcGlcAs efficiently rescued the tumor-suppressive function of mutant p53. (A) Luciferase reporter assay of p53-V272M transcriptional activity on the *PUMA* promoter in H1299 cells. Cells were treated with AcGlcAs or ATO for 1 h, followed by culture in fresh medium for 24 h. (B) RT-qPCR analysis of mRNA levels of the indicated p53 target genes in U937 cells overexpressing p53-V272M after ATO or AcGlcAs treatment for 1 h and culture in fresh medium for another 4 h. (C) Western blot analysis of the protein levels of the indicated p53 targets in U937 cells overexpressing p53-V272M after ATO or AcGlcAs treatment for 1 h and culture in fresh medium for another 16 h. (D) RT-qPCR analysis of mRNA levels of the indicated p53 targets in U937 cells overexpressing p53-V272M. (E, F) The cell cycle profiles of U937 cells expressing p53-V272M were determined by PI staining and flow cytometry. Representative profiles are shown in panel (E). Cells were treated with ATO or AcGlcAs for 1 h and cultured in fresh medium for another 24 h. (G, H) Apoptosis analysis of U937 cells overexpressing p53-V272M by Annexin V-FITC and 7-AAD staining using flow cytometry. Representative profiles are shown in panel (G). The treatment method was the same as that in panel (E). (I, J) Colony formation of U937 cells overexpressing p53-V272M. Cells were treated with ATO or AcGlcAs for 1 h and then plated in fresh soft agar medium. Representative profiles are shown in panel (I). Colonies were counted on day 14 (J). (K–N) RNA-seq analysis. (K) Top three enriched NCI nature pathways and MSigDB Hallmark pathways for the upregulated differentially expressed genes (DEGs; fold change >2 , $P < 0.05$) in U937 cells harboring p53-V272M after ATO or AcGlcAs treatment at the indicated concentrations for 1 h and culture in fresh medium for another 4 h. The P value for each gene was calculated using the edgeR package, which was applied without sample replicates. (L) Heatmap of the relative mRNA levels of the reported 116 p53 target genes in V272M cells, normalized to the DMSO sample. (M) Quantitative PCR determination of the *CDKN1A*, *PUMA*, and *MDM2* mRNA expression. (N) Volcano plot showing the distribution of expression changes of p53 target genes. Red dots represent p53 target genes.

established experimental approach was employed, wherein active cellular uptake pathways were selectively obstructed through incubation at 4 °C.^{48–50} As compared to the standard 37 °C incubation, 4 °C incubation dramatically impaired the

cellular uptake of As upon both 1 and 10 μ M AcGlcAs treatment in HepG2 cells (Figure 3K), suggesting that an active cellular uptake pathway, presumably the thiol-mediated pathway, is the major cell uptake pathway of AcGlcAs.

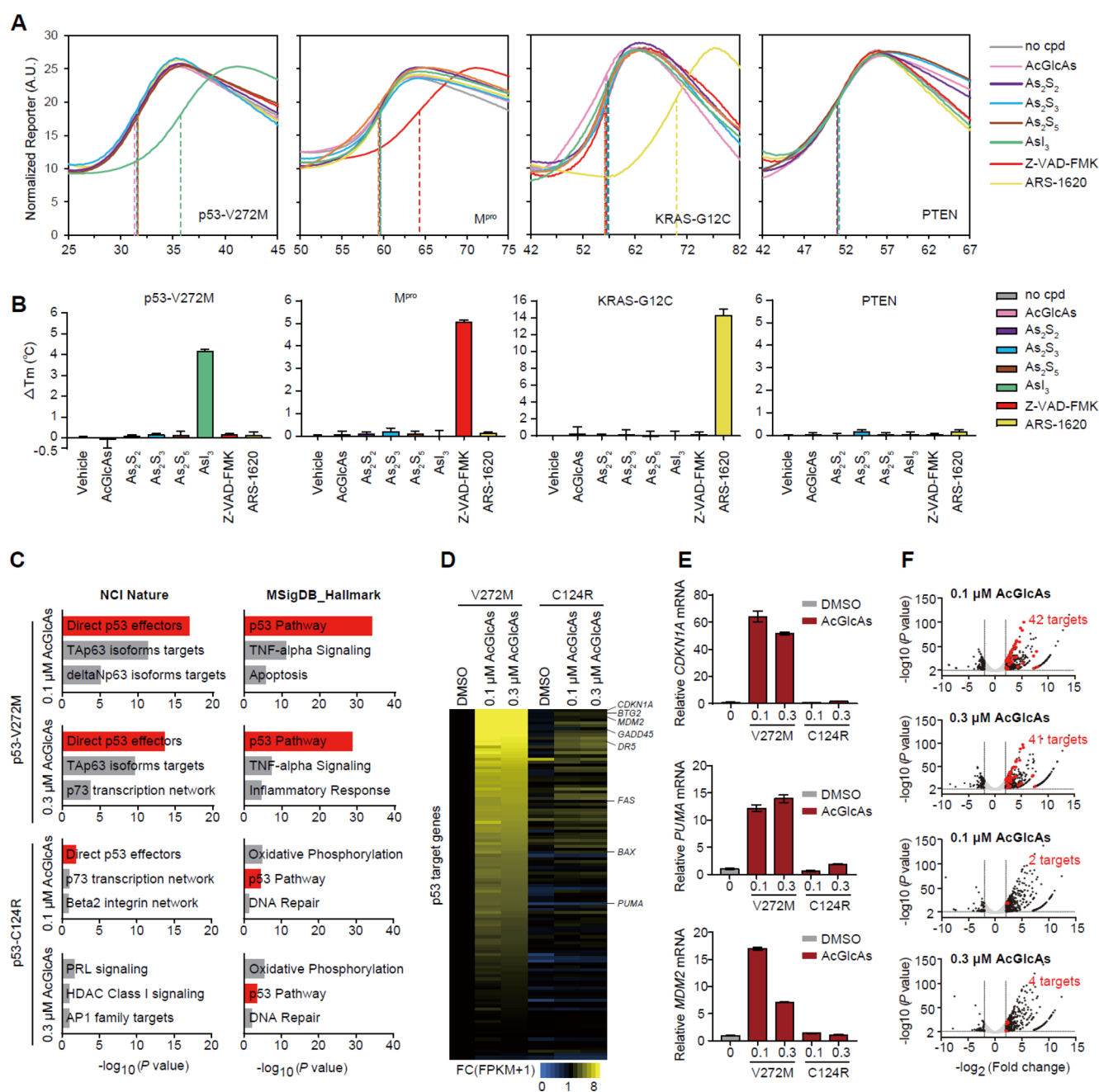


Figure 6. Target specificity of AcGlcAs. (A) Recombinant p53-V272M DBDs, SARS-Cov-2M^{Pro}, KRAS-G12C, and PTEN, were instantly incubated with the indicated compounds in a ratio of 1:15 in 20 mM HEPES (pH 7.5). Melting curves were measured by DSF. (B) Calculated ΔT_m based on the data in panel (A). (C–F) RNA-seq analysis. (C) Top three enriched NCI nature pathways and MSigDB Hallmark pathways for the upregulated DEGs (fold change >2, $P < 0.05$) in U937 cells harboring p53-V272M or p53-C124R after AcGlcAs treatment at the indicated concentrations for 1 h and culture in fresh medium for another 4 h. The P value for each gene was calculated using the edgeR package, which was applied without sample replicates. (D) Heatmap of the relative mRNA levels of the reported 116 p53 target genes in V272M or C124R cells, normalized to the DMSO sample. (E) Quantitative PCR determination of the *CDKN1A*, *PUMA*, and *MDM2* mRNA expression. (F) Volcano plot showing the distribution of expression changes of p53 target genes. Red dots represent p53 target genes.

AcGlcAs Induced Potent Degradation of Oncogenic PML/RAR α . We next validated the potency of AcGlcAs in inducing the degradation of the APL-specific oncoprotein PML/RAR α . At equimolar As concentrations, AcGlcAs was much more potent in inducing the degradation of PML/RAR α than ATO after 1 h of treatment in both NB4 cells and PML/RAR α -overexpressing 293T cells (Figure 4A and Figure S6). The results of immunofluorescence staining supported the high potency of AcGlcAs in degrading PML/RAR α , showing a

decrease in micropunctuates and appearance of large PML speckles characteristic of normal nuclear bodies in most treated cells⁵¹ (Figure 4B and Figure S7). Consequently, the well-identified PML/RAR α -activating genes in NB4,⁵² including *P2RY2*, *DLK1*, *MPO*, and *FCGR1B*, were effectively down-regulated by AcGlcAs in a dose-dependent manner (Figure 4C).

We next determined the cell fate triggered by AcGlcAs treatment. It was reported that PML/RAR α degradation induced by ATO leads to differentiation and apoptosis of NB4

cells.⁵³ Indeed, 3 and 9 μM AcGlcAs respectively induced a partial granulocytic differentiation in 32.9 and 20.9% of NB4 cells, according to the cell surface differentiation marker CD11b (Figure 4D,E), which was much more potent than ATO. Moreover, AcGlcAs showed high potencies in inducing apoptosis (Figure 4F,G). Upon treatment with 27 μM AcGlcAs, 43.8% of the cells became apoptotic, a much higher proportion than that upon ATO treatment. Consequently, AcGlcAs efficiently reduced the colony-formation ability of NB4 cells (Figure S8). Taken together, AcGlcAs exhibited a high potency in degrading PML/RAR α .

AcGlcAs Efficiently Rescued the Tumor-Suppressive Function of Mutant p53. We further determined the efficiency of AcGlcAs in rescuing the structural mutant p53. In the luciferase reporter assay, 0.3–10 μM AcGlcAs significantly promoted the transcriptional activity of p53-V272M on PUMA upon 1 h or 10 min treatment (Figure 5A and Figure S9). Importantly, AcGlcAs was much more potent than ATO in rescuing p53-V272M at equimolar As concentrations (Figure 5A; 10 μM As, 112.2- vs 2.2-fold). Consistent with the transactivation of p53-V272M, AcGlcAs dramatically upregulated the representative p53 targets p21 (encoded by *CDKN1A*), PUMA, and MDM2 at both the mRNA and protein levels in U937 (Figure 5B,C) and other tested cell lines (Figures S10 and S11). The canonical p53 triggered cell fates including apoptosis, cell cycle arrest, and senescence.⁵⁴ Furthermore, apoptosis can be triggered through either the caspase-mediated extrinsic or intrinsic pathway. To better understand the therapeutic mechanism of AcGlcAs, a series of direct p53 target genes involved in the above three cell fates were analyzed by RT-qPCR, including cell cycle arrest-promoting genes *CDKN1A* (encodes p21), *GADD45A*, and *BTG2*; intrinsic apoptosis-promoting genes *PUMA* and *BAX*; extrinsic apoptosis-promoting genes *FAS*, *DR5*, and *PERP*; and senescence-promoting genes *CDKN1A* and *PML*. All of the tested p53 target genes, except *PML* and *PERP*, showed significant upregulation in U937 cells harboring p53-V272M after AcGlcAs treatment (Figure 5D). These results imply that the AcGlcAs-reactivated mutant p53 possibly kills cancer cells through multiple pathways.

We next determined the cell fates triggered by AcGlcAs-reactivated mutant p53. It was found that the cell cycle was dramatically arrested upon AcGlcAs treatment in U937 cells harboring p53-V272M (Figure 5E,F), which is in line with the observed upregulation of cell cycle arrest-promoting targets *CDKN1A*, *GADD45A*, and *BTG2* (Figure 5B,D). In the PI staining experiment, AcGlcAs treatment increased the percentage of both early and late apoptotic cells dramatically in U937 cells harboring p53-V272M (Figure 5G,H). It is possible that both intrinsic apoptosis and extrinsic apoptosis are triggered by AcGlcAs since both of the p53 target genes involved in these two types of apoptosis are upregulated by AcGlcAs (Figure 5B,D). Further, we did not observe significantly increased levels of SA- β -gal-positive cells (senescence cells) upon AcGlcAs treatments (Figure S12). This is partly consistent with the defect of AcGlcAs-reactivated mutant p53 in the upregulating senescence-promoting p53 target gene *PML* (Figure 5D). As a result, AcGlcAs dramatically reduced the colony-formation ability of U937, HL60, and H1299 cells stably expressing p53-V272M (Figure S1J and Figures S13 and S14; 8 μM As in U937 cells, 14 vs 175 colonies).

We further performed RNA-seq in U937 cells harboring arsenic-rescuable p53-V272M upon AcGlcAs and ATO treat-

ments (Figure 5K and Table S2). After 0.1 or 0.3 μM AcGlcAs treatments for 1 h, the differential expression genes (DEGs; fold change >2 or <0.5, $P < 0.05$) upregulated by compound treatment were most enriched in “Direct p53 effectors” in the NCI-Nature database and “p53 Pathway” in MSigDB_Hallmark databases (Figure 5K, red bars, $P = 1.6 \times 10^{-17}$ and 1.4×10^{-34} for 0.1 μM AcGlcAs, respectively; $P = 2.8 \times 10^{-14}$ and 1.8×10^{-29} for 0.3 μM AcGlcAs, respectively). The DEGs upregulated by ATO treatment at equimolar As concentrations were also enriched in p53 pathway-related terms but to a notably lesser extent compared to AcGlcAs treatment. We next focused on the panel of the 116 established p53 target genes.⁵⁵ In the heatmap of p53 targets, a substantial number of these representative p53 targets, including the set of the focused pro-apoptotic genes (Figure 5B,D), exhibited a high level of upregulation in response to AcGlcAs treatment, whereas they were only moderately upregulated by ATO (Figure 5L, validated by qPCR in Figure 5M). In the volcano plots, there were only 3–5 p53 targets that are highly upregulated by ATO at As concentrations of 0.1 and 0.3 μM (Figure 5N, red dots). However, there were up to 41–42 p53 targets that were highly upregulated by AcGlcAs at equimolar As concentrations. Taken together, AcGlcAs was highly potent in rescuing structural mutant p53 through multiple pathways including apoptosis.

AcGlcAs Specifically Targets Mutant p53. We next evaluated the targeting specificity between AcGlcAs and mutant p53 using the *in vitro* differential scanning fluorimetry (DSF) assay and the RNA-seq assay in cells. In the DSF assay, an assay in which a purified recombinant protein will be thermostabilized upon binding to a small molecule,⁵⁶ AcGlcAs failed to thermostabilize the p53 DBD-binding domain (DBD) containing an arsenic atom-binding pocket (Figure 6A,B; no increase in melting temperature). This can be explained by AcGlcAs possessing relatively stable As–S bonds within the DSF buffer, thereby impeding the release of As³⁺ ions required for binding to p53. Consistently, there is no binding between the p53 DBD and other As–S bond-containing arsenicals (Figure 6A,B; As₂S₂, As₂S₃, and As₄S₄) in the solution. These results are in line with our previous findings that in the DSF assay, the similar As–O bond of ATO was stable and thus could not instantly thermostabilize p53-V272M DBD.⁵⁷ We thus seek to apply p53-reactivating AsI₃ (Figure 1), which likely contains the As³⁺ ion in the AsI₃ solution. As anticipated, AsI₃ exhibited an immediate and potent thermostabilization effect on p53 in this assay (Figure 6A,B; with the melting temperature increased by 4.2 °C). Interestingly, AsI₃ cannot bind a set of recombinant proteins containing exposed cysteine, including SARS-Cov-2M^{pro} with exposed Cys145,⁵⁸ KRAS-G12C with exposed Cys12,⁵⁹ and PTEN with exposed Cys124. As positive controls, the reported M^{pro}-targeting compound Z-VAD-FMK specifically binds M^{pro}, while the reported KRAS-G12C-targeting compound ARS-1620 specifically binds KRAS-G12C.

In the RNA-seq assay in U937 cells harboring arsenic-rescuable p53-V272M⁶⁰ (Figure 6C–F and Table S2), the DEGs that were upregulated by 0.1 and 0.3 μM AcGlcAs treatment were predominantly enriched in “Direct p53 effectors” in the NCI-Nature database and “p53 Pathway” in MSigDB_Hallmark databases (Figure 6C, red bars, $P = 1.6 \times 10^{-17}$ and 1.4×10^{-34} for 0.1 μM AcGlcAs, respectively; $P = 2.8 \times 10^{-14}$ and 1.8×10^{-29} for 0.3 μM AcGlcAs, respectively). Indeed, a larger number of the 116 established p53 targets were upregulated by AcGlcAs (Figure 6D–F). To ensure that the observed upregulation of p53 target genes is indeed caused by

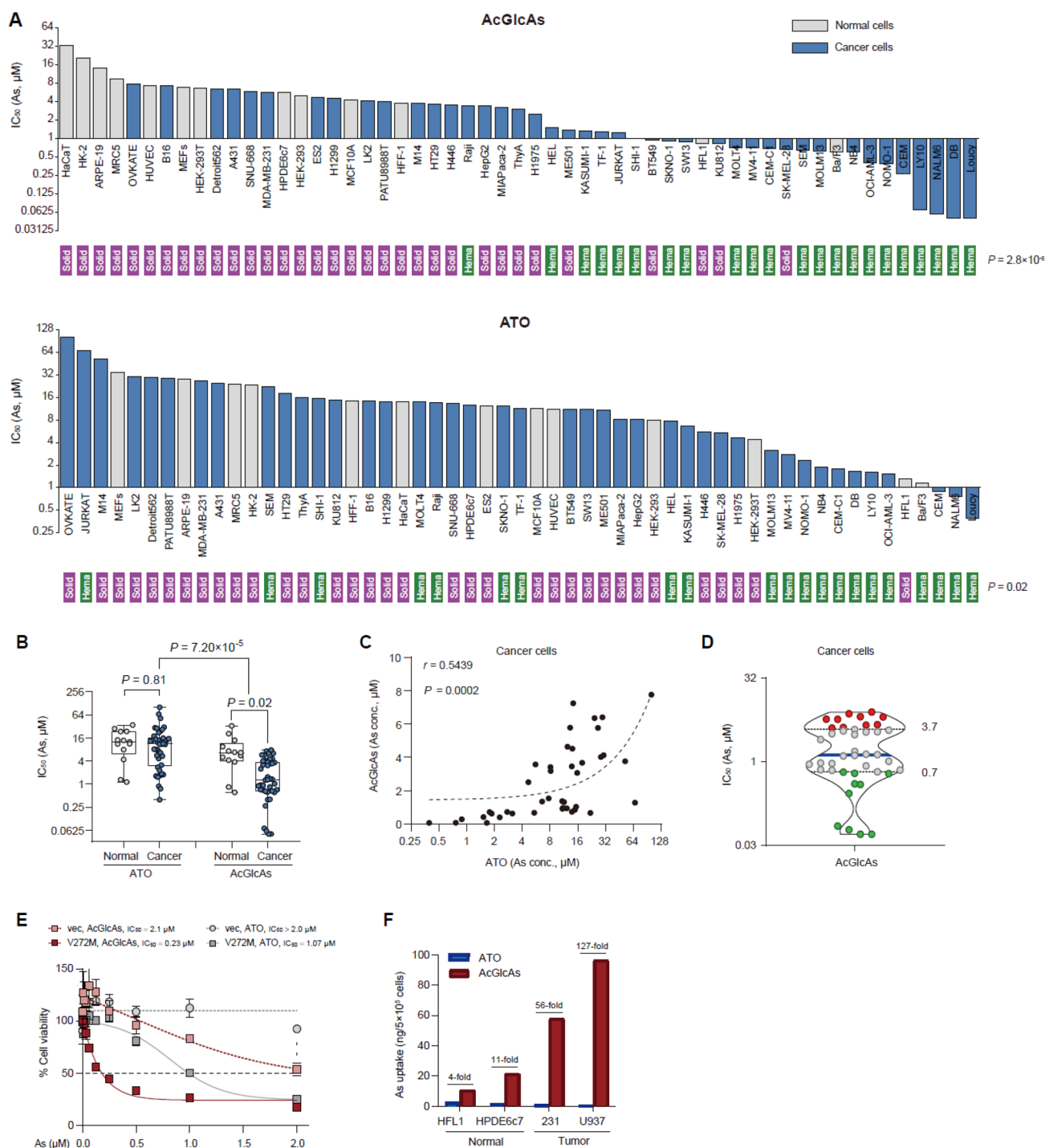


Figure 7. AcGlcAs potently and selectively inhibited cancer cell proliferation. (A) Bar chart showing the IC_{50} distribution of AcGlcAs and ATO in 13 normal cell lines and 42 cancer cell lines. Viabilities of cell lines, upon ATO or AcGlcAs treatment for 48 h, were determined by CCK-8 assay. Solid, cell lines derived from solid tissues; Hema, hematological cell lines. P values show the difference between IC_{50} values for solid-tumor cell lines and hematological malignant cell lines. (B) Box plot showing IC_{50} values of AcGlcAs and ATO for the 55 tested cell lines. The statistical significance was determined using the unpaired, two-tailed Student's t -test. (C) Scatter plot showing associations between IC_{50} values of AcGlcAs and ATO on the 42 cancer cell lines. (D) Violin plot of IC_{50} values of AcGlcAs on the 42 cancer cell lines. The first and third quartiles of IC_{50} are shown. (E) Cell viability of U937 cells infected with an empty vector or p53-V272M upon ATO or AcGlcAs treatment for 48 h, as determined by CCK-8 assay. (F) Cellular As contents in the indicated normal and cancer cell lines upon ATO or AcGlcAs treatment (10 μM As) for 10 min, as determined by ICP-MS. Data of MDA-MB-231 and U937 cells are from Figure 3A.

binding of As to p53-V272M, we further applied an arsenic-binding defected p53-C124R mutant, in which one cysteine of the arsenic-binding pocket is mutated,^{10,60} as a control in RNA-seq. Consequently, the DEGs upregulated by both 0.1 and 0.3

μM AcGlcAs in the p53-C124R cells are poorly enriched in the p53-related pathway (Figure 6C). Furthermore, only a limited number of p53 targets are upregulated by AcGlcAs in p53-C124R cells compared to p53-V272M cells (Figure 6D–F).

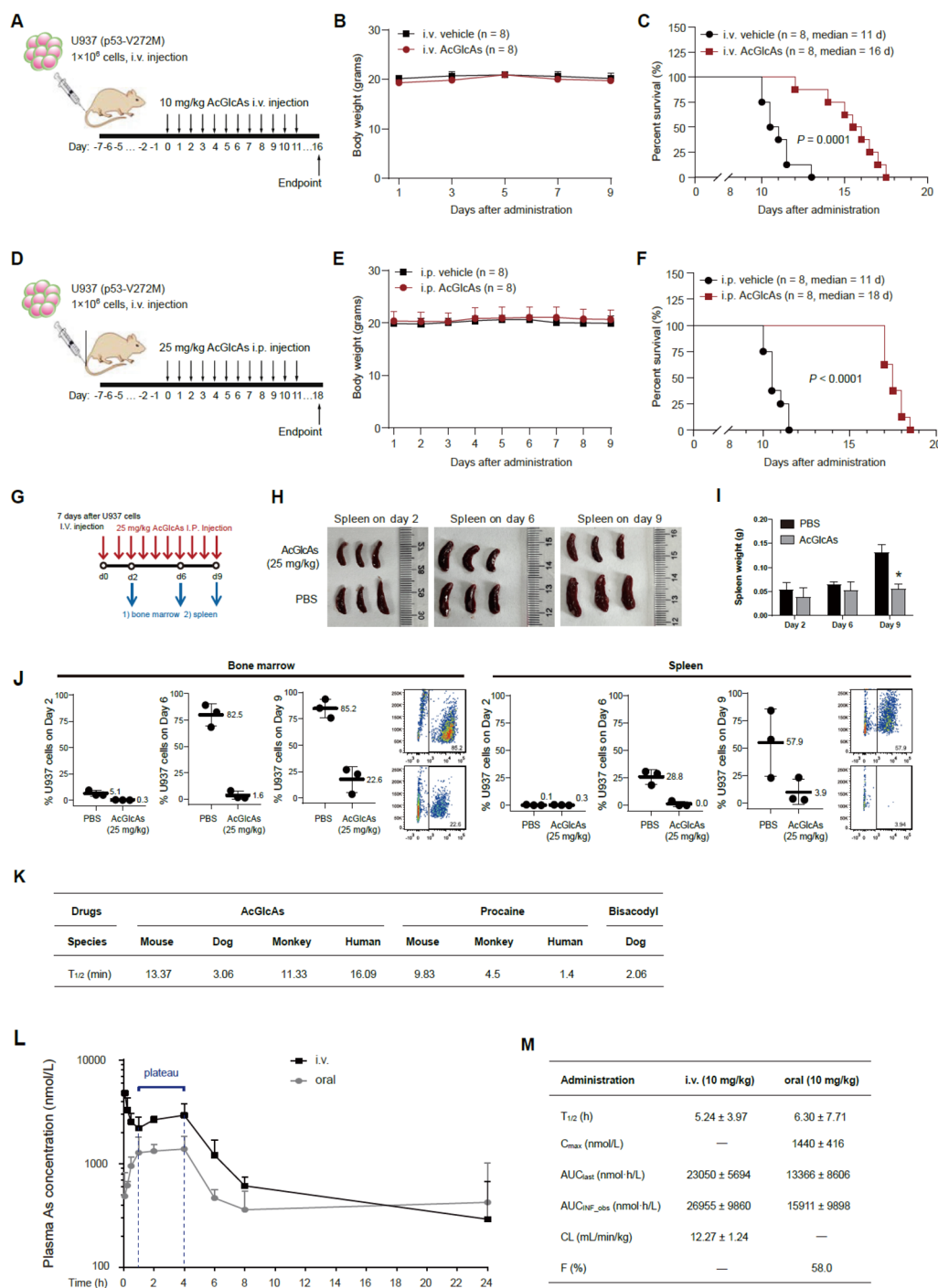


Figure 8. AcGlcAs in preclinical mouse models. (A–C) Toxicity and anticancer activity of AcGlcAs in mice with i.v. administration of 10 mg/kg AcGlcAs. (A) Experimental procedure. (B) Toxicity analysis by monitoring body weight after administration of AcGlcAs (mean $\pm$ SD, $n = 8$). (C) Kaplan–Meier survival curves of the mice. (D–F) Toxicity and anticancer activity of AcGlcAs in mice with i.p. administration of 25 mg/kg AcGlcAs. (D) Experimental procedure. (E) Toxicity analysis by monitoring body weight after administration of AcGlcAs (mean $\pm$ SD, $n = 8$). (F) Kaplan–Meier survival curves of the mice. (G, J) Xenograft mouse model derived from U937 cells expressing p53-V272M. (G) M-NSG mice were inoculated with GFP-labeled U937 cells expressing p53-V272M through the tail vein and treated with 25 mg/kg AcGlcAs daily from day 7 post inoculation. (H, I) Photograph (H) and weight (I) of the isolated spleen. (J) Proportion of leukemia U937 cells in BM and spleen on days 2, 6, and 9, as determined by FACS based on GFP fluorescence (mean $\pm$ SD, $n = 3$). The representative FACS profiles of the samples isolated on day 9 are shown in the right panel. See also Figure S17. (K) *In vitro* plasma stability of AcGlcAs. (L) Plasma As concentration–time curves of mice after i.v. or oral administration of AcGlcAs at the dose of 10 mg/kg, as determined by ICP-MS ($n = 3$). (M) Preliminary PK evaluation of As in mice after the administration of 10 mg/kg AcGlcAs.

These results in V272M and C124C isogenic cell lines strongly suggest that AcGlcAs exhibits a high degree of binding specificity toward the C124-C135-C141 pocket of p53.

Together, both DSF and RNA-seq results suggested that mutant p53 is the predominant target of AcGlcAs. Such target specificity can be explained by the fact that arsenic has a strong

binding preference to trithiol rather than monothiol or dithiol.⁶¹ Since the number of proteins containing a cysteine triad (three spatially proximate cysteine residues) is much smaller than the number of proteins containing cysteine in the cells, the arsenic atom expectedly exhibits target specificity toward p53 in the cells.

AcGlcAs Potently and Selectively Inhibited Cancer Cell Proliferation. Since cancer cells generally have a higher level of cell-surface thiol than nontransformed cells,^{29–32} we speculated that AcGlcAs might potently and selectively kill cancer cells. A panel of normal and cancer cell lines was then collected for AcGlcAs cytotoxic analysis. To make the cytotoxic results more comparable among cell lines with genetic heterogeneity, we collected up to 55 cell lines (Table S3). Cells were treated with AcGlcAs or ATO for 48 h, followed by IC₅₀ determination using the CCK8 assay (Figure 7A; raw cell growth curves seen in Figure S15). In the IC₅₀ profile, AcGlcAs had <1 μM IC₅₀ in about half of the tested cancer cell lines while ATO in only three cancer cell lines (Figure 7A, blue bars under the *x*-axis). Indeed, AcGlcAs killed the panel of cancer cell lines much more potently than ATO (Figure 7B, median values, 1.32 vs 11.22 μM ; $P = 7.20 \times 10^{-5}$), presumably due to the efficient thiol-coupling uptake of AcGlcAs. In addition, in the profile of AcGlcAs, normal cells apparently clustered at the left (Figure 7A, gray bars), implying the preference of AcGlcAs in inhibiting cancer cells. Statistically, AcGlcAs has significantly high cytotoxicity in the 42 cancer cell lines as compared to the 8 normal cell lines (Figure 7B, median values, 1.32 vs 6.62 μM , $P = 0.02$). In contrast, ATO had almost completely equal IC₅₀ values between the cancer cell lines and normal cell lines (Figure 7B, median values, 11.22 vs 12.68 μM , $P = 0.81$). This supports the cancer cell selectivity of AcGlcAs, presumably due to the reported higher level of cell-surface thiol in cancer cells. Interestingly, we further noticed that hematological cell lines, in general, exhibited greater sensitivity to AcGlcAs (as well as ATO) treatment when compared to cell lines derived from solid tissues (Figure 7A; $P = 2.8 \times 10^{-5}$). The underlying mechanism remains to be further studied.

We next looked into the IC₅₀ diversity of AcGlcAs and ATO among the 42 tested cancer cell lines. First, a strong positive correlation was observed between the IC₅₀ values of AcGlcAs and ATO (Figure 7C, $r = 0.54$, $P = 0.0002$). This correlation indicated that AcGlcAs and ATO shared a similar mechanism of action (MoA), likely involving the targeting of some protein(s) in the cells. Second, AcGlcAs displayed overall lower IC₅₀ values than ATO, supporting its enhanced cellular uptake efficiency. Regarding IC₅₀ diversity among the 42 IC₅₀ values, the IC₅₀ at the third quartile was 5.3 times higher than that at the first quartile (Figure 7D, 3.7 and 0.7 μM , respectively). The diversity of the AcGlcAs IC₅₀ values is possibly caused by the different genetic backgrounds of the tested 42 cell lines. Indeed, a variety of the widely used anticancer drugs including cisplatin, etoposide, paclitaxel, and vinblastine also exhibit diverse IC₅₀ values in inhibiting the same set of the 42 cancer cell lines (Figure S16; the IC₅₀ values at the third quartile were 3.6, 6.7, 8.1, and 4.3 times higher than those at the first quartile for the four anticancer drugs, respectively; data were derived from the Cancerxgene database⁶²).

The isogenic cell-line model is an ideal model to correlate cytotoxicity to a gene mutation. We introduced the p53-null U937 cell line with structural p53 mutant V272M or empty vector. The resultant cell lines have identical genetic backgrounds (except for the introduced p53 mutant), and in theory,

any cytotoxicity difference observed can be attributed to the introduced p53 mutant. As a result, introduction of V272M strongly conferred cells' sensitivity to AcGlcAs (Figure 7E, IC₅₀ values 0.23 vs 2.1 μM). Introduction of V272M also conferred U937 cells' sensitivity to ATO (IC₅₀ values 1.07 vs >2 μM). This suggested that the cytotoxicities of both AcGlcAs and ATO were largely dependent on structural p53 mutants. In addition, AcGlcAs is more cytotoxic than ATO in U937 cells harboring V272M (IC₅₀ values 0.23 vs 1.07 μM), consistently supporting the potent thiol-coupling uptake of AcGlcAs.

To directly test whether the observed cell inhibition potency and cancer cell selectivity of AcGlcAs are attributed primarily to efficient uptake by cells and selective uptake by cancer cells, respectively, we detected the cellular uptake of As in four representative cell lines upon ATO and AcGlcAs treatment. As expected, AcGlcAs was more efficient in permeating into cells than ATO in all four tested cell lines (Figure 7F, red bars vs blue bars). Importantly, AcGlcAs was more efficient in permeating into cancer cells than normal cells (Figure 7F, see the labeled fold changes). Taken together, AcGlcAs potently and selectively inhibited cancer cells, particularly those with structural p53 mutations.

AcGlcAs Exhibited High Druggability in Preclinical Mouse Models. To determine the *in vivo* anticancer activity of AcGlcAs, we intravenously injected the luciferase-transduced leukemia cell line U937 harboring p53-V272M into M-NSG mice to generate a xenograft model. Seven days after xenografting, 10 mg/kg AcGlcAs was intravenously injected into mice daily (Figure 8A). No obvious toxicity was induced by AcGlcAs administration in mice, as reflected by the unaltered body weight (Figure 8B). Notably, AcGlcAs significantly extended the median survival of xenografted mice from 11 to 16 days after administration (Figure 8C, $P = 0.0001$). In a parallel experiment, tumor progression was monitored by bioluminescence imaging (BLI) of the luciferase signal (Figure S17A), and a significant delay of U937 expansion was observed upon AcGlcAs administration (Figures S17B), explaining the survival benefit provided by AcGlcAs treatment (Figure 8C). We also tested the anticancer activity of intraperitoneally injected AcGlcAs (Figure 8D). It was found that the median survival was extended from 11 to 18 days after AcGlcAs administration without dramatic body weight changes (Figure 8E,F, $P < 0.0001$). We further generated a xenograft model by intravenously injecting mice with the GFP-labeled U937 harboring p53-V272M. Seven days after xenografting, 25 mg/kg AcGlcAs or PBS was intraperitoneally injected into mice daily, followed by mice sacrifice upon 2, 6, and 9 day drug treatment (Figure 8G). Leukemia cells frequently enrich in the spleen, leading to its swelling. Interestingly, the isolated spleens in the AcGlcAs group are apparently smaller and lighter than the ones in the PBS group on days 2 and 6, however, without statistical significance (Figure 8H,I). However, on day 9, AcGlcAs treatment significantly delayed the swelling of the spleen (Figure 8H,I). We further determined the percentage of leukemia U937 cells in isolated bone marrow and spleen. AcGlcAs treatment decreased the population of U937 cells in both bone marrow and spleen at all of the three end points (Figure 8J, days 2, 6, and 9). Together, AcGlcAs not only extended the survival of the xenograft mice (Figure 8A–F) but also effectively slowed leukemia cell expansion during the treatment process (Figure 8G–J).

We further evaluated the PK properties, oral bioavailability, and biodistribution of AcGlcAs. In the *in vitro* plasma stability

assay in mouse, dog, monkey, and human, AcGlcAs displayed half-lives of 13.37, 3.06, 11.33, and 16.09 min, respectively (Figure 8K, procaine and bisacodyl served as controls). PK parameters of AcGlcAs in mice were acquired by determining As concentration after single intravenous (i.v.) and intragastric (i.g., oral) injections of 10 mg/kg AcGlcAs (Figure 8L,M). In the plasma concentration–time profiles, mice intravenously injected with AcGlcAs exhibited a relatively high and stable level of plasma As between 1 and 4 h after i.v. and oral administration (Figure 8L, plateau), with $T_{1/2}$ values of 5.24 and 6.30 h (Figure 8M), respectively. Compared to the reported relatively short half-life of ATO in humans (1.0–2.0 h for i.v. and 6.0 h for oral administrations), rats (about 3.7 h for i.v. administration), and mice (about 4.3 h for i.v. administration),^{15,16,63,64} the determined half-life of AcGlcAs is promising. The plasma clearance (CL) of AcGlcAs in i.v.-injected mice was 12.3 mL/min/kg (Figure 8M), which was 13.7% of the hepatic blood flow of 90 mL/min/kg in mice,⁵¹ indicating a good metabolic stability of AcGlcAs. In addition, the oral bioavailability of AcGlcAs in mice was calculated to be 58% (Figure 8M), suggesting that oral administration may be a feasible, although not the most optimal, administration modality for AcGlcAs. In addition, after single i.v. administration of 10 mg/kg AcGlcAs, arsenic was rapidly distributed into different tissues, predominantly accumulating in the liver, kidneys, and lungs (Figure S19 and Tables S4 and S5), consistent with the tissue affinities of arsenic in a reported ATO study.⁶⁵ In most organs except the bladder, arsenic was eliminated within 8 h. This could be explained by the rapid excretion of arsenic via the kidneys into the bladder.⁶⁶ Generally, AcGlcAs displayed high druggability with significant anticancer activity, good plasma stability, and acceptable oral bioavailability.

CONCLUSIONS

In our analysis with the aim to develop a novel p53-targeting arsenical, we revealed that a highly stable As–C bond was associated with poor activity in targeting PML/RAR α and mutant p53, in line with the reported mechanism of action of ATO.^{4,10} The revealed SAR is particularly valuable for arsenic-based anticancer therapy, since it potentially establishes a guideline for future development of novel arsenicals with p53-targeting activity. The revealed SAR, combined with the proposed strategy of thiol-mediated As delivery, enabled us to develop the novel AcGlcAs, which not only retains PML/RAR α - and p53-targeting activities but also exhibits efficient cellular uptake and cancer cell selectivity. In detail, the uptake of AcGlcAs in cancer cell lines was much more efficient than the one of ATO (Figure 7F, 56–127-fold improvement) and, consequently, the potency of AcGlcAs in inhibiting cancer cell lines was significantly higher than the one of ATO (Figure 7B, $P = 7.20 \times 10^{-5}$). In addition, AcGlcAs was preferentially uptaken by cancer cell lines rather than normal cell lines (Figure 7F, 56–127-fold vs 4–11-fold) and, consequently, AcGlcAs selectively inhibited cancer cell lines rather than normal cell lines (Figure 7B, $P = 0.02$). Mechanistically, ATO enters cancer cells by hijacking the nonspecific transporter like AQP9,¹⁴ with limited efficiency. By contrast, AcGlcAs is able to make use of abundant cell-surface thiols to efficiently enter cells and, thus, AcGlcAs is more potent in targeting PML/RAR α or mutant p53 to kill cancer cells than ATO (Figure S20, left panel vs middle panel). Since cancer cells frequently have more abundant cell-surface thiols than normal cells,^{29–32} AcGlcAs displayed a preference in

killing cancer cells rather than normal cells, as hoped (Figure S20, middle panel vs right panel).

It is worth noting that the efficiency of the cellular uptake of AcGlcAs can be up to 127-fold higher than that of ATO. This efficiency is much higher than even that of the reported promising As@Fn, which displayed an approximately 4.5-fold increase of As uptake compared to ATO.²⁴ In addition, in the presence of structural mutant p53, we observed that the non-APL cancer cell lines, such as U937, were strikingly sensitive to AcGlcAs and were even more sensitive than the representative APL cell line NB4 is to the clinical ATO concentrations (arsenic IC₅₀, 0.23 vs 1.86 μ M). Given the miraculous efficacy of ATO in treating APL at safe dosages in the human body, it is reasonable to expect a promising efficacy of AcGlcAs in the treatment of cancers with structural mutant p53.

Overall, our study not only provides a framework for rationally optimizing structure of anticancer arsenicals but also supplies a druggable molecular entity that could be used to treat the large population of patients with p53-mutated cancers.

EXPERIMENTAL SECTION

Chemical Reagents. For the 12 arsenicals (ATO and arsenicals 1–11), ATO (arsenic trioxide), NaAsO₂ (sodium arsenite), As₂O₅ (arsenic pentoxide), As₂S₂ (arsenic(II) sulfide), As₂S₅ (arsenic pentasulfide), AsCl₃ (arsenous trichloride), AsI₃ (arsenous triiodide), C₆H₅AsNO₃ (arsanilic acid), and C₆H₅AsO (phenylarsine oxide) were purchased from Sigma, As₂S₃ (arsenic(III) sulfide) was purchased from Macklin, KAsF₆ (potassium hexafluoroarsenate) was purchased from Alfa Aesar, and C₁₆H₁₀AsN₂Na₃O₁₁S₂ (Arsenazo I) was purchased from Sangon. The key intermediates 2,3,4,6-*O*-tetraacetyl thioglucose (compound 12) and 2-chloro-1,3-dithia-2-arsacyclopentane (compound 13) for AcGlcAs were prepared in Prof. Xin Cao's lab according to the reference methods.^{42,43} Primary antibodies used for western blotting or immunofluorescence analysis were purchased from the following companies: Flag (Sigma), β -actin (Genscript), RAR α (Santa Cruz), PML (Santa Cruz), DO1 (detecting p53, Abcam), GAPDH (Cell Signaling Technology), p21 (Cell Signaling Technology), PUMA (Cell Signaling Technology), and MDM2 (Sigma). The secondary antibodies antimouse IgG HRP-linked and antirabbit IgG HRP-linked antibodies were purchased from Cell Signaling Technology. The antimouse IgG (H+L) highly cross-adsorbed secondary antibody Alexa Fluor 488 was purchased from Invitrogen. Other reagents for western blotting were purchased from the following companies: Tris-MOPS-SDS Running Buffer Powder (Genscript), and Express Plus PAGE Gel (Genscript). Primary antibodies or reagents used in FACS were purchased from the following companies: anti-CD11b-FITC (Beckman Coulter), 7-AAD (BD Pharmingen), FITC Annexin V Apoptosis Detection Kit I (BD Pharmingen), propidium iodide (PI, Sigma), and RNase A (Sangon). For inhibitors, *N*-ethylmaleimide (NEM) was purchased from Sigma, methyl- β -cyclodextrin (M β CD) and chlorpromazine (CPZ) were purchased from MedChemExpress, and Auranofin was purchased from Topscience. For reagents used for RT-qPCR, the Total RNA Purification Kit was purchased from Sangon, and HiScript II QRT SuperMix for qPCR (+ gDNA wiper) and ChamQ SYBR qPCR Master Mix were purchased from Vazyme. For reagents used for cell culture, PBS buffer (BasalMedia), DMEM, 1640 medium, Opti-MEM medium, and penicillin–streptomycin were purchased from Thermo Fisher, Ham's F-12K (Kaighn's) medium was purchased from Gibco, and fetal bovine serum (FBS) was purchased from Moredgate Biotech. Other reagents or kits were purchased from the following companies: Fluoroshield Mounting Medium With DAPI (Abcam), Cell Counting Kit-8 (TargetMol), FuGENE6 transfection reagent kit (Promega), HilyMax transfection reagent kit (Dojindo), Dual Luciferase Reporter Assay Kit (Vazyme), Complete Protease inhibitor cocktail (Sigma), Wright-Giemsa staining solution (BASO), D-Luciferin Potassium Salt (YEASEN), Fluoroshield Mounting Medium with DAPI (Abcam), DMSO (Meilunbio), Cremophor EL (CREL, Macklin), Agarose Low

melting (Sangon), and crystal violet staining solution (Sangon). Distilled water was purified with a Millipore Milli-Q Biocel purification system.

Instruments. Electrospray ionization mass spectrometry (ESI-MS) spectra were recorded by using Agilent 1260 liquid chromatography (LC)–mass spectrometry (MS). High-resolution electrospray ionization mass spectrometry (HRMS-ESI) spectra were recorded using an Agilent HRMS. UV–vis spectra were recorded with a Varian Cary 100 UV/vis spectrophotometer (USA). ^1H NMR and ^{13}C -NMR spectra were recorded on a Bruker Avance 500 spectrometer. The X-ray crystal structure assay was recorded and characterized with PLATON. The As content was recorded using an inductively coupled plasma mass spectrometer iCAPQ (Thermo). Western blotting was carried on EPS 300, VE 180, and VE 586 (Tanon), and western blotting imaging was monitored with an Amersham Imager 600 (GE Healthcare Life Sciences). Fluorescence confocal imaging was monitored with a Leica sp8 confocal microscope Leica sp8. Cell viabilities were analyzed using a Varioskan Flash (Thermo). Luciferase reporter assay was analyzed using a SpectraMax L (Molecular Devices). Flow cytometric analysis was carried out using an LSRFortessa™ X-20 instrument (BD Biosciences). RT-qPCR was carried out on a ViiA 7 Real-Time PCR System (Applied Biosystems). Bioluminescence was done using an IVIS Spectrum In Vivo Imaging System (PerkinElmer).

Synthetic Procedures and Characterization of AcGlcAs. All of the reactions were carried out under nitrogen or argon with anhydrous solvents in flame-dried glassware, unless otherwise noted. The chemicals used were of reagent grade as supplied, except where noted. In detail, the solution of tetraacetyl- β -D-thioglucofuranose (12, 364 mg, 1 mmol)⁴² in 1 mL of CHCl_3 was added to 2-chloro-1,3-dithia-2-arsacyclopentane (13, 202 mg, 1 mmol)⁴³ in 3 mL of CHCl_3 with 1:1 molar ratio. The mixture was heated at reflux for 6 h. Then, the mixture was diluted with 10 mL of CH_2Cl_2 and washed with brine for two times. The organic layer was extracted and dried over Na_2SO_4 for 2 h. Then, it was filtered, and the filtrate was concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (petroleum ether/EtOAc, 4:1) to provide the white solid product at 291 mg (0.55 mmol) with 55% yield. ^1H NMR (500 MHz, CDCl_3) δ 5.22–5.06 (m, 3H), 4.74 (d, J = 9.4 Hz, 1H), 4.20 (ddd, J = 14.8, 12.4, 3.7 Hz, 2H), 3.76 (ddd, J = 9.9, 4.9, 2.5 Hz, 1H), 3.58–3.32 (m, 4H), 2.10 (s, 3H), 2.04 (s, 3H), 2.02 (s, 3H), 1.99 (s, 3H). ^{13}C NMR (126 MHz, CDCl_3) δ 170.57, 170.11, 169.28, 169.18, 82.93, 76.26, 73.60, 72.04, 68.12, 61.91, 42.74, 42.70, 20.78, 20.74, 20.56, 20.53; ESI (+)-MS, 553.25 $[\text{M} + \text{Na}]^+$. HRMS-ESI (m/z): $[\text{M} + \text{Na}]^+$ calculated for $\text{C}_{16}\text{H}_{23}\text{AsO}_9\text{S}_3\text{Na}$, 552.9616 (theoretically calculated mass: 552.9618). Anal. ($\text{C}_{16}\text{H}_{23}\text{AsO}_9\text{S}_3$) C, H, N. The X-ray crystal structures of AcGlcAs were analyzed using X-ray crystallography and stored in Cambridge Crystallographic Data Centre (CCDC) with the accession code CCDC 2122393. The purity of AcGlcAs is >95%, as confirmed by NMR.

Chemical Stability Tests for AcGlcAs. To determine reactivity between AcGlcAs and NEM, AcGlcAs (10 μM) was incubated with NEM (100 μM) in PBS for 1 h at room temperature, accompanied by LC-MS (Agilent 6100) detection at 0 and 60 min, respectively, to confirm whether AcGlcAs reacts with NEM. However, there was no new peak and MS monitored except for AcGlcAs and NEM. After that, the mixture was recovered by extraction and checked by ^1H NMR. There was no sign for new compounds according to the comparison of parallel ^1H NMR spectra between standards and the recovered drugs (Figure 3E). To determine the stability of AcGlcAs, AcGlcAs (10 μM) was incubated in PBS for 12 h with LC-MS detection afterward, while no obvious new peak and MS were detected. After that, the drug was recovered by extraction and confirmed by ^1H NMR. The results suggest that AcGlcAs is stable enough in PBS solution for at least 12 h.

Cell Source and Culture Conditions. NB4 cells were a gift from Tan et al.⁵² A431, B16, BT549, CEM, CEM-C1, DB, Detroit562, ES2, H1299, HepG2, HL60, HPDE6c7, HT29, HUVEC, JURKAT, KASUMI-1, KU812, Loucy, M14, ME501, MIApaca-2, MOLM13, MOLT4, MV4-11, NALM6, NOMO-1, OVKATE, Raji, SK-MEL-28, SNU-668, SW13, TF-1, U937, HK-2, MCF10A, HaCaT, HFF-1, and HEK-293T were purchased from the American Type Culture

Collection (ATCC). ARPE-19, HEK-293, HEL, HFL1, H446, H1975, LK2, MDA-MB-231, and MRC5 were purchased from the Cell Bank of the Chinese Academy of Sciences. Ba/F3, LY10, OCI-AML-3, PATU8988T, SEM, SHI-1, and SKNO-1 cells were obtained from the Leibniz Institute DSMZ Cell Bank. The stable cell lines U937, HL60, and H1299 overexpressing p53-V272M, as well as the stable cell line H1299 harboring p53-V272M, luciferase, and Renilla, were maintained in our lab. MEFs (C57BL/6 mouse embryonic fibroblasts) and ThyA (a cell line derived from the thymic lymphoma of p53^{R279W/+} mice) were prepared and maintained in our lab. All the adherent cells, except MEFs, HUVEC, HFL1, HT29, and SK-MEL-28, were maintained in DMEM with 10% (v/v) FBS, 100 U/mL penicillin, and 100 mg/mL streptomycin. MEFs were maintained in DMEM with 10% (v/v) FBS, 1% pyruvate sodium (Gibco), 1% NEAA (Gibco), 1% GlutaMAX, penicillin, and streptomycin. HUVECs and all of the suspension cells except Ba/F3 were maintained in RPMI 1640 medium with 10% FBS, penicillin, and streptomycin. HFL1 cells were maintained in F-12K Nutrient Mixture with 10% FBS, streptomycin, and penicillin. HT29 cells were maintained in McCoy's 5A with 10% FBS, streptomycin, and penicillin. SK-MEL-28 cells were maintained in MEM with 10% FBS, streptomycin, and penicillin. Ba/F3 was maintained in RPMI 1640 with 10% vol conditioned medium of the W3 (WEHI-3B) cell line. All cell lines were maintained in a humidified atmosphere at 37 °C with 5% CO_2 .

Mice. M-NSG mice (NOD-Prkdc^{scid}IL2rg^{em1}/Smoc) were purchased from the Shanghai Model Organisms Center, Inc., and were housed under specific pathogen-free conditions. Experiments were carried out according to a Shanghai Jiao Tong University IACUC-approved protocol. The Institute of Cancer Research (ICR) male mice were purchased from Shanghai Jie Si Jie Laboratory Animal Co., Ltd. (<http://www.jsj-lab.com/>). The use of mice was approved by the Animal Ethics Committee of the School of Medicine, Shanghai Jiaotong University, and the approval number is A2019004. All animal care protocols and experiments were reviewed and approved by the Animal Use Committee of the School of Medicine, Shanghai Jiaotong University.

Cell Proliferation Assay. Proliferation assay was performed using Cell Counting Kit-8 (CCK-8) in accordance with the manufacturer's instructions. Briefly, cells were plated at a density of 5000 cells/well in 96-well plates, and there were three replicates for each sample. The arsenicals at various concentrations were added to each well for 48 h of incubation. A cell culture medium without arsenicals was added to the control wells, and wells without cells were used as blank controls. Dye solution was added, and the plates were incubated at 37 °C for 3–4 h before the absorbance was detected at 450 nm. IC_{50} and AUC values were calculated by GraphPad Prism 8.

Western Blot Analysis. Western blot was performed as reported previously.⁶⁷ Briefly, cells were lysed in a urea lysis buffer. Protein extracts were loaded onto SDS polyacrylamide gels. Gels were transferred onto nitrocellulose membranes, and the resulting blots each were incubated with a primary antibody and then an appropriate HRP-conjugated secondary antibody, followed by HRP activity-based signal detection.

The PML/RAR α degradation determination in NB4 cells and 293T cells overexpressing the protein was performed as reported previously.⁶⁸ The cell samples were washed twice with PBS buffer, followed by the lysis of the cell pellets using RIPA lysis buffer (50 mM Tris–HCl, pH 7.5, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 0.2 mM PMSF, and a complete miniprotease inhibitor tablet). Whole cell lysates (L) were incubated on ice for 10 min with vortexing in 5 min intervals and centrifuged for 30 min at 13,000g at 4 °C to obtain the supernatants (S) for western blot analysis. Pellet fractions (P) were washed twice with PBS and then lysed in LDS buffer (1 $\times$ TBS, 10% glycerol, 0.015% EDTA, 50 mM DTT, and 2% SDS), and boiled for 10 min at 95 °C. Then, the PML/RAR α proteins were quantified using the same protocol mentioned above.

Luciferase Reporter Assay. To analyze the effects of the 11 arsenicals and ATO on p53-V272M transcriptional activity on the PUMA promoter, H1299 cells stably overexpressing p53-V272M were seeded in 100 μL of medium at 50% density in 96-well plates. After 24 h,

the arsenicals diluted in 50 μ L of room-temperature medium were added to each well. After another 24 h, cells were lysed, followed by luciferase signal determination using a luciferase assay kit. The luminescence signal of firefly luciferase was normalized against that of Renilla luciferase for each sample. To analyze the effects of the 11 arsenicals and ATO on p53-V272M transcriptional activity on the *CDKN1A* promoter, cells were seeded in 100 μ L of medium at 30% density in 96-well plates for 24 h. To do the transfection for 10 wells, 3 μ L of FuGENE transfection reagent and 50 μ L of Opti-MEM were mixed and incubated for 5 min and then added to the premixed three plasmids (750 ng of p53-expressing plasmid, 250 ng of luciferase reporter plasmid, and 12 ng of Renilla plasmid). After 24 h, the cells were treated with ATO and the 11 arsenicals using the same method as the above-mentioned.

To determine the influence of short-time (10 min or 1 h) treatment with AcGlcAs and ATO on the transcriptional activity of p53-V272M on the *PUMA* promoter, the stable cell line H1299 harboring p53-V272M, luciferase, and Renilla was seeded in 100 μ L of medium at 50% density in 96-well plates. After 24 h, cells were incubated with 100 μ L of fresh medium containing AcGlcAs at the indicated concentrations. Upon incubation at 37 $^{\circ}$ C for 10 min, the cells were washed once with PBS and exchanged with 100 μ L of fresh medium for 24 h of incubation for luciferase signal determination. To determine the effects of inhibitors on AcGlcAs, the cells were pretreated with Auranofin, N-ethylmaleimide (NEM), methyl- β -cyclodextrin (M β CD), or chlorpromazine (CPZ) at the indicated concentrations for 30 min, followed by incubation with AcGlcAs for 10 min and fresh-medium culture for another 24 h.

Cellular Arsenic Uptake. The cellular uptake of arsenic was measured on HepG2, HFL1, HPDE6c7, MDA-MB-231, and U937 (p53-V272M) cells. The cells were seeded in 6 cm cell-culture dishes overnight and then incubated with ATO or AcGlcAs at the equimolar arsenic concentration (10 μ M) under standard culture conditions for 10 or 60 min. Then, the cells were washed with PBS buffer three times and harvested by trypsinization. The numbers of harvested cells were counted manually. After that, the cells were concentrated and digested by nitric acid for inductively coupled plasma mass spectrometry (ICP-MS).

The subcellular localization of As in HepG2 was determined by ICP-MS. The cells were treated with 10 μ M AcGlcAs for 1 h, then washed with PBS three times, and harvested by trypsinization. The nucleus and cytoplasm isolation was performed as previously described.⁶⁹

The influence of NEM on AcGlcAs in the cellular uptake of arsenic was also determined by ICP-MS. HepG2 cells were pretreated with 0 or 100 μ M NEM for 30 min and subsequently incubated with 10 μ M AcGlcAs for 10 min. Then, the arsenic contents of cells were quantified using the same protocol mentioned above.

Knockdown of Target Genes. The H1299 cells stable expressing p53-V272 M were transfected with siRNA using RFECT siRNA transfection reagent (Biotrans, China) following the recommended procedures. The siRNA sequences are as follows: siTFRC-1 sense: 5'-AACUUCAGGUUUCUGCCAGCdTdT-3'; siTFRC-1 antisense: 5'-GCUGGCAGAAACCUUGAAGUdTT-3'; siTFRC-2 sense: 5'-GGUUGCAAAGCUGCAAAGCdTdT-3'; siTFRC-2 antisense: 5'-GCUUUCAGCAUUGCAACCDdT-3'; siCKAP4 sense: 5'-GCAGGAUUUGAAAGCCUUAAdTdT-3'; siCKAP4 antisense: 5'-UUUAAGGCUUCAAUCCUGCdTdT-3'; siSCARB1 sense: 5'-GCAAGGUUGACUUCUGGCAUdTT-3'; siSCARB1 antisense: 5'-AAUGCCAGUAGUACCUUGCdTdT-3'; siCLIC1 sense: 5'-GUGGAUGAAACCAGUGCUGAAAdTdT-3'; siCLIC1 antisense: 5'-UUCAGCACUGGUUUAUCCACdTdT-3'.

Real-Time qPCR. Total RNA was isolated from cells using a Total RNA Purification Kit. Then, 1 μ g of total RNA was reverse-transcribed using the HiScript II QRT SuperMix for qPCR (+ gDNA wiper) following the manufacturer's protocol. PCR was performed in triplicate using a ChamQ SYBR qPCR Master Mix (Low ROX Premixed) and ViiA 7 Real-Time PCR System under the following conditions: 10 min at 95 $^{\circ}$ C, followed by 40 cycles of 95 $^{\circ}$ C for 15 s and 60 $^{\circ}$ C for 45 s. Specificities of the PCR products were checked for each primer set and sample via melting curve analysis. Expression levels of genes were

normalized relative to levels of β -actin by adopting the comparative C_t method. The primer sequences are as follows: P2RY2_F: 5'-CACCCCTCAACGCCATCAACA-3'; P2RY2_R: 5'-TAC-GAGCCTCTGCCAGC-3'; *DLK1*_F: 5'-GCAACCCCCAAAATG-GATTCT-3'; *DLK1*_R: 5'-GGTACGCACTGGTCACAAA-3'; *MPO*_F: 5'-GGCTCTAGACCTGCTGGAGAG-3'; *MPO*_R: 5'-CGGTGATGGTGCGGTATTTG-3'; *FCGR1B*_F: 5'-GAAATCCA-CAGAGGCTGGCTAC-3'; *FCGR1B*_R: 5'-AGTGGAAAACT-TAAAGGCTTTGCC-3'.

Immunofluorescence Analysis. NB4 cells were treated with ATO or AcGlcAs for 1 h, followed by fresh-medium culture for 0.5, 6, and 24 h. NB4 cells untreated were used as a control (0 h). The cells were harvested and washed with PBS, and then 1×10^5 cells were centrifuged into the glass slides by cytospin at 800 rpm for 5 min. Subsequently, cells were fixed in 4% phosphate-buffered saline (PBS)–paraformaldehyde for 15 min, incubated in 0.2% Triton X-100 for 5 min and then 1% BSA in PBS for 10 min, and stained for 1 h with PML antibody. Staining with a secondary antibody was performed for 20 min, followed by three washes. After that, the cells were shielded by Fluoroshield Mounting Medium with DAPI and then visualized under a Leica sp8 fluorescence confocal microscope Leica sp8.

The 293T cells were grown on cover glasses in a 24-well plate, and the Flag-PML/RAR α plasmid was transfected into 293T cells. Twenty-four hours after transfection, the cells were treated with ATO or AcGlcAs at the equimolar arsenic concentration (As, 3 μ M) for 1 h. Then, the cells were washed once with PBS and exchanged with 1 mL of fresh medium for 24 h of incubation for immunofluorescence analysis.

Colony Formation Assay. For the cell line H1299 stably expressing p53-V272M, about 1000 cells/well were seeded in triplicate in a 12-well plate. After 24 h of culture, the cells were treated with ATO or AcGlcAs. Upon incubation at 37 $^{\circ}$ C for 1 h, the cells were washed once with PBS and then exchanged with 1 mL of fresh medium for 7 days, during which period the medium was not changed. Cells were then washed with PBS and stained with crystal violet. ImageJ2x was used for statistics of the area of colonies.

The colony formation assay of U937 and HL60 cells stably expressing p53-V272M was performed as previously described with minor changes.⁷⁰ Briefly, cells were treated with ATO or AcGlcAs for 1 h. After that, the cells were harvested and rinsed once in PBS to completely remove the residual media. Then, about 2000 cells/well were seeded on the 0.35% top agarose overlaid onto solidified 0.7% agarose in RPMI 1640 containing 10% FBS in 12-well plates. Cultures were fed every week. Colonies were stained with crystal violet and analyzed at the time points indicated in figure legends. The colony number was counted by using ImageJ2x.

Wright-Giemsa Staining. NB4 cells were treated with ATO or AcGlcAs at the indicated concentrations for 1 h, followed by fresh-medium culture for 48 h. After that, the untreated cells were harvested and washed with PBS. Then, 1×10^5 cells were centrifuged into glass slides by cytospin at 800 rpm for 5 min. The glass slides were dried and stained in the Wright-Giemsa staining solution according to the manufacturer's instruction.

Flow Cytometry Analysis. Cells were treated with ATO or AcGlcAs as the figure legends indicated. For granulocytic differentiation, apoptosis, and cell-cycle arrest assays, 1×10^5 cells were washed with PBS and incubated with CD11b-FITC or 7-AAD-PE/Annexin V-APC for 15 min. For cell cycle analysis, 1×10^6 cells were washed with PBS and fixed with 70% precool ethanol overnight at -20° C. Next, fixed cells were centrifuged, washed once with PBS, and resuspended in 350 μ L PI staining solution (50 μ g/mL PI, 0.1% Triton X-100, 50 μ g/mL RNase A, in PBS) for 20 min at 4 $^{\circ}$ C. Then, cells were detected using an RSII (BD Biosciences) and analyzed by FlowJo software (version 10).

Senescence Assay. Cell senescence was determined using an SA- β -Gal staining kit (Beyotime, C0602) according to the manufacturer's protocol. Briefly, U937 cells overexpressing p53-V272M were plated in six-well plates at 50% confluence per well and treated with AcGlcAs or ATO at indicated concentrations for 1 h, followed by fresh-medium culture for 8 h. After being washed with PBS, cells were fixed at room temperature for 15 min. After washing, 1 mL SA- β gal solution was

added to each well, and cells were incubated at 37 °C overnight. Cells were photographed by using an electron microscope.

RNA Sequencing. Total RNA was isolated from cells using a Total RNA Purification Kit (B518651, Sangon Biotech). Sequencing libraries were constructed from 1 µg of purified mRNA using an Illumina TruSeq RNA Sample Prep Kit (cat. #FC-122-1001). Libraries were pooled and 150 bp paired-end reads were sequenced on an Illumina Novaseq 6000 platform. Sequencing service was provided by GENEWIZ Biotech Co. (Suzhou, China). For data analysis, raw sequence reads were initially processed using FastQC (Babraham Institute) for quality control. Adapter sequences and poor-quality reads were removed using Cutadapt. Quality-filtered reads were then mapped to the human genome using Hisat2, and gene-level read counts were generated from aligned reads by HTSeq (v0.6.1). Differential gene expression analysis was carried out using R (v4.1.0) edgeR package (v3.30.3), setting the dispersion value to calculate for the sample with no replicates. The volcano plot and heatmap were generated using R with ggplot2 (v3.3.3) and pheatmap (v1.0.12), respectively. For gene set enrichment analysis, the upregulated DEGs (fold change >2 and *P* value <0.05) were input into Enrichr (<https://amp.pharm.mssm.edu/Enrichr/>) using default settings, which returned the enriched terms.

Differential Scanning Fluorimetry. Differential scanning fluorimetry (DSF) assay was performed as reported.⁵⁷ Briefly, 3 µM purified recombinant p53-V272M DBD, SARS-Cov-2M^{Pro}, KRAS-G12C, or PTEN was mixed with the indicated compounds at the final molar ratio of 1:15 in 20 mM HEPES (pH 7.5), followed by immediate melting temperature determination.

In Vitro Plasma Stability Assay. Potassium phosphate buffer (0.1 M) was preheated with PBS at pH 7.4 ± 0.1 to 37 °C. The frozen plasma was thawed by placing at a 37 °C water bath quickly. Ten microliters of 10 mM AcGlcAs or reference compounds procaine and bisacodyl stock solution was added to 990 µL of DMSO, and 99 µL of prewarmed plasma was added into the wells designated for all the time points (0, 5, 15, 30, 60, and 120 min). Then, 1 µL of prewarmed spiking solution was added into the 99 µL plasma of the wells designated for all the time points. Afterward, 400 µL of MeOH:CAN (1:1) containing the internal standard (IS) was added into the corresponding wells of the 0 min plate to stop the reaction immediately. Then, 400 µL of MeOH:CAN (1:1) containing IS was added into the wells of corresponding plates at 5, 15, 30, 60, and 120 min. After such a quenching procedure, the plates were shaken for 5 min (600 rpm) and stored at −20 °C. After all the samples were collected, these samples were thawed at RT and centrifuged at 6000 rpm for 20 min, and 100 µL of the supernatant from each well was transferred into a 96-well sample plate containing 100 µL of ultrapure water. Finally, all samples were analyzed with LC-MS.

Pharmacokinetic and Biodistribution Study. For pharmacokinetic study, male ICR mice (*n* = 3 per group; weight, 25–30 g; age, 6–8 weeks) were treated with AcGlcAs at the dose of 10 mg/kg via intravenous (i.v.) and intragastric (i.g., oral) injection. Blood samples were collected at 0.083, 0.25, 0.5, 1, 2, 4, 8, and 24 h after administration. Plasma samples were obtained through common procedures, and the concentrations of arsenic in the supernatant were analyzed by ICP-MS.

For the biodistribution study, male ICR mice (*n* = 5 per group) were treated with AcGlcAs at the dose of 10 mg/kg via intravenous injection. The major organs or tissues (bladder, brain, heart, kidney, liver, lung, spleen, skin, and testis) were collected at 0.5, 2, 8, and 24 h after administration. After tissues were rinsed with saline, excess liquid was removed with filter paper, and the mixture was then weighed. All samples were stored at −70 °C until analysis using ICP-MS.

Mouse Xenograft Model. U937 cells infected with the MigR1 vector containing p53-V272M (MigR1-V272M-IRES-eGFP) were sorted by flow cytometry and expanded in culture, and 1 × 10⁶ cells were injected intravenously into the tail vein of 7 week-old female M-NSG mice. Seven days following tumor cell injection, mice were randomized into two groups (8 mice per group) and then injected intravenously or intraperitoneally with vehicle or AcGlcAs daily as indicated in the figure legends. The 1 mg/mL AcGlcAs solution was prepared by dissolving AcGlcAs powder in the 2:6:92 (vol/vol/vol)

DMSO:CrEL:PBS mixture right before injection. The body weight was recorded manually. The lifespan of mice was monitored every 12 h and analyzed by Log-rank (Mantel-Cox) test.

For monitoring tumor progression by bioluminescence imaging (BLI) for the luciferase signal, the U937 cells overexpressing p53-V272M were further infected with the MigR1 vector containing firefly luciferase gene (MigR1-LUC-IRES-RFP). The cells were sorted using flow cytometry and expanded in culture, and 1 × 10⁶ cells were injected intravenously into the tail vein of 7 week-old female M-NSG mice. The procedure about how the mice were treated with vehicle or AcGlcAs and when their bioluminescence was detected is indicated in the figure legend. To detect the bioluminescence, the luciferase substrate D-luciferin firefly potassium salt at a dose of 150 mg/kg was administered by intraperitoneal injection. The mice were then anesthetized by 1.5–2.5% isoflurane inhalation, and bioluminescence was done 10–15 min later using an IVIS Spectrum In Vivo Imaging System in the Instrumental Analysis Center, Shanghai Jiao Tong University. Total bioluminescence was quantified for the body area that included each mouse in its entirety using Living Image (64-bit).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.jmedchem.3c00104>.

Additional figures illustrating the effects of the 12 arsenicals on the degradation of PML/RAR α , SAR summary of the 12 arsenicals, NMR data of AcGlcAs, effect of AcGlcAs on the degradation of PML/RAR α in 293T cells, colony formation assay, luciferase reporter assay, RT-qPCR analysis, and western blotting analysis of AcGlcAs in other cell lines; dose–response curves for inhibition of cell viability by AcGlcAs in 55 cell lines; bioluminescence images of the tumor burden in the mice administered AcGlcAs; biodistribution of As in main organs following i.v. administration of 10 mg/kg AcGlcAs in male ICR mice; schematic illustration of the cellular uptake and anticancer mechanism of AcGlcAs (PDF)

RNA-seq data of U937 cells harboring p53-V272M or p53-C124R upon AcGlcAs treatment (XLSX)

Molecular string of AcGlcAs (CSV)

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Notes

The authors declare the following competing financial interest(s): Min Lu, Ying Liang and Shujun Xiao are co-authors of the pending patents Multifunctional p53 Rescue Compounds and Their Uses (CN202110659536.4).

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ABBREVIATIONS

ATO, arsenic trioxide; APL, acute promyelocytic leukemia; As, arsenic; PK, pharmacokinetics; SAR, structure–activity relationship; TATG, tetraacetyl- β -D-thioglucose; CMP, cell membrane protein; ESI-HRMS, high-resolution electrospray ionization mass spectrometry; ICP-MS, inductively coupled plasma mass spectrometry; NEM, N-ethylmaleimide; M β CD, methyl- β -cyclodextrin; CPZ, chlorpromazine; BLI, bioluminescence imaging; i.v., intravenous injection; i.g., intragastric injection; CL, plasma clearance; TLC, thin-layer chromatography

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