Abstract

A major challenge to the treatment of advanced prostate cancer (PCa) is the development of resistance to androgen-deprivation therapy (ADT) and chemotherapy. It is imperative to discover effective therapies to overcome drug resistance and improve clinical outcomes. We have developed a novel class of silicon-containing compounds and evaluated the anticancer activities and mechanism of action using cellular and animal models of drug-resistant PCa. Five organosilicon compounds were evaluated for their anticancer activities in the NCI-60 panel and established drug-resistant PCa cell lines. GH1504 exhibited potent in vitro cytotoxicity in a broad spectrum of human cancer cells, including PCa cells refractory to ADT and chemotherapy. Molecular studies identified several potential targets of GH1504, most notably androgen receptor (AR), AR variant 7 (AR-v7) and survivin. Mechanistically, GH1504 may promote the protein turnover of AR, AR-v7 and survivin, thereby inducing apoptosis in ADT-resistant and chemoresistant PCa cells. Animal studies demonstrated that GH1504 effectively inhibited the in vivo growth of ADT-resistant CWR22Rv1 and chemoresistant C4-2B-TaxR xenografts in subcutaneous and intrareouscous models. These preclinical results indicated that GH1504 is a promising lead that can be further developed as a novel therapy for drug-resistant PCa.

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Keywords: Prostate cancer, Castration-resistance, Chemoresistance, Small-molecule therapy, Silicon-containing compounds, Preclinical studies

Abbreviations: ADT, androgen deprivation therapy; AMDP(OEt)3, 1-aminoethylhexadiphosphonic acid tetrachyl ester amide residue; AR, androgen receptor; AR-V7, AR variant 7; Atmp, 4-amino-1,2,6,6-tetramethylpiperidine amide residue; BmSimob, 4-[(butyldimethylsilyl)methoxy]-benzoyl; Bip, β-(4-biphenylyl)alanine residue; BOP, benzotriazol-1-yl-oxytris(dimethylamino)phosphonium hexafluorophosphate; COX-2, cyclooxygenase-2; CRPC, castration-resistant prostate cancer; DlEA, N,N-disopropylethylamine; GRP78, glucose-regulated protein 78kD; HSP27, heat shock protein 27; HSP90, heat shock protein 90; IGF-1R, insulin-like growth factor-1 receptor; IHC, immunohistochemistry; KIF15, Kinesin family member 15; LBD, ligand-binding domain; MSipob, 4-[(trimethylsilyl)propoxy]-benzoyl; NTD, N-terminal domain; OC2Y, O-2,6-dichlorobenzyl-tyrosine residue; PCa, prostate cancer; PROTAC, proteolysis-targeting chimera; PSA, prostate-specific antigen; PyBOP, benzotriazol-1-yl-oxytris(dipyrrolidino)phosphonium hexafluorophosphate; SIAH2, siah E3 ubiquitin protein ligase 2; TFA, trifluoroacetic acid.

* Corresponding author at: Center for Cancer Research and Therapeutic Development and Department of Biological Sciences, Clark Atlanta University, Atlanta, GA, USA.

E-mail address: dwu@cau.edu (D. Wu).

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Introduction

Prostate cancer (PCa) is the most commonly diagnosed cancer and the second leading cause of cancer deaths in American men [1]. In 2021, an estimated 248,530 new cases are expected, and 34,130 patients will die [2]. Although most patients with localized PCa respond to standard treatments, including surgery, radiation therapy and first-generation androgen-deprivation therapy (ADT), in many cases PCa continues to progress towards castration resistance (CRPC) [3]. Docetaxel was approved in 2004 as a first-line chemotherapy for metastatic CRPC, although it only demonstrated a limited survival advantage [4]. Since 2010, the FDA has approved several new medications for the treatment of CRPC, mainly the new-generation chemotherapy (cabazitaxel) and ADT (abiraterone acetate, enzalutamide, apalutamid, and darolutamide) [5]. In recent Phase III clinical trials, such as STAMPEDE and CHAARTED, the combination of docetaxel and standard ADT (“chemohormonal” therapy) demonstrated prolonged overall survival compared to single chemotherapy or ADT in patients with advanced PCa [6,7]. Unfortunately, CRPC remains a dreadful evolution of PCa and eventually develops extreme resistance to ADT, chemotherapy, or both. Clearly, it is an urgent and unmet medical need to develop effective treatments to overcome therapeutic resistance and improve the survival and quality of life of CRPC patients.

At the molecular levels, CRPC is characterized by a significant rewiring of various oncogenic signaling pathways that allow cancer cells to evade their apoptotic fate and survive, despite drug treatments [8–10]. Research over the last decade has confirmed that the reactivation of the androgen receptor (AR) signaling is likely the most important molecular event during CRPC progression; this can be achieved via multiple mechanisms, including the amplification, mutation and alternative splicing of the AR gene [3,11–14]. AR overexpression could be identified in up to 80% of CRPC specimens, whereas hormone-naïve tumors rarely exhibit AR amplification [13]. AR variant 7 (AR-v7), the best-characterized splicing variant so far, lacks the C-terminal ligand-binding domain (LBD) of full-length AR but retains an N-terminal domain (NTD); therefore it is capable of inducing androgen-independent AR transactivation [15]. Several lipid biopsy methods for AR-v7 detection have been established, and the occurrence and upregulation of AR-v7 in circulating tumor cells have been correlated to acquired resistance to abiraterone and enzalutamide in CRPC patients [16–20]. In preclinical models, both AR- and AR-v7-dependent transcriptional complementation are required for resistance to the second-generation ADT [21,22], and small-molecule inhibition of AR-v7 could sensitize CRPC cells to ADT treatment [23–27]. These observations indicated that the activation of AR-v7 expression and signaling might play an important role in ADT resistance, and AR-v7 represents a promising target to overcome therapeutic resistance [22].

Several mechanisms of chemoresistance have been proposed [8]. Rare subpopulations of cancer cells with stemness or neuroendocrine characteristics may be responsible for intrinsic chemoresistance [28], and overexpression of drug efflux pumps (such as the ATP-binding cassette transporter p-glycoprotein) may be pivotal in acquiring chemoresistance [29,30]. Deregulation of apoptotic pathways has been implicated in both chemoresistance and ADT resistance. Previous studies from us and others demonstrated that overexpression of survivin, a classic oncosignal gene that is differentially expressed in normal and cancer cells, promotes PCa progression and confers therapeutic resistance [31–34].

The complex biology and high heterogeneity of advanced cancers indicated that a targeted therapy aimed at a single molecule or pathway would be less effective, and novel agents that can simultaneously inhibit/block multiple oncogenic signals (such as AR, AR-v7 and survivin) should be developed to overcome therapeutic resistance [35]. Molecular hybridization, a medicinal chemistry strategy that combines two or more pharmacophores into a single chemical entity, has been recognized as a promising approach to develop novel drug candidates with higher efficacy through multiple-target interaction [36,37]. During the past decade, we have designed several generations of small molecules that have a common three-component (“A-B-C”) structure consisting of three distinct pharmacophores, with the expectation of co-targeting multiple oncogenic factors and achieving better anticancer activities. Several compounds with biphenylalanine (Bip) or O-(2,6-dichlorobenzyl)-L-tyrosine (OC2Y) residues at the “B” position, including BKM570, BKM1644, BKM1740 and BKM1972, demonstrated promising efficacy in preclinical models of PCa and other cancer types [38–47]. Recently, we reported that LG1836, a molecule containing an o-phenylcinnamic acid derivative as the “A” component and OC2Y as the “B” component, effectively inhibited the in vitro and in vivo growth of CRPC via the suppression of AR, AR-v7 and survivin [48]. These results warrant further efforts to develop more efficacious compounds that can be used to treat drug-resistant PCa.

Replacing a carbon atom with silicon, or the Sila-substitution (C/Si-exchange) of existing drugs, has been actively explored as an effective approach in the search for new biochemically active small molecules [49–52]. Carbon and silicon present some key differences in term of atomic size, bond length, electronegativity and lipophilicity. These differences can lead to changes in how the carbon and silicon analogues interact with their targets, thus resulting in different physico-chemistry and pharmacology between the two types of compounds [52]. For example, a silicon analogue is generally more lipophilic than its carbon equivalent, which may markedly increase tissue penetration and cellular uptake of the compound, thereby having improved in vivo stability and anticancer activity [53]. Bzikhanova et al. reported that the addition of a silicon to indomethacin, an anti-inflammatory drug, resulted in silica-indomethacin, or “silamethacin”. Compared with the parent compound, the silicon derivative retained its function as a selective cyclooxygenase-2 (COX-2) inhibitor while demonstrating enhanced potency in human cancer cells [54,55]. Similarly, the introduction of a (trimethylsilyl)ethyl group into camptothecin, a DNA topoisomerase inhibitor, significantly improved its anticancer activity in a broad spectrum of human cancer cells, including multi-drug resistant cells. Compared to camptothecins, the lipophilic silicon-containing Karenitecin (BNP1350) exhibited enhanced tissue penetration and bioavailability [56], and has entered clinical trials in melanoma, glioma and lung cancer. Inspired by the “C/Si-exchange” concept for rational drug design and built upon our earlier generations of anticancer compounds, we developed a novel class of silicon-containing small molecules and determined their potential for the treatment of CRPC. In this report, we described one of such compounds, namely GH1504, that exhibited promising efficacy against both ADT-resistant and chemoresistant PCa in preclinical models.

Materials and methods

Chemical synthesis

Silicon-containing compounds with a three-component (“A-B-C”) structure were synthesized following the methods that we described previously [46,57]. The “A” component 3-(4-butyl-1H-indol-3-yl)benzoic acid (CAS: 948301-32-4) or 4-[butyl-dimethylsilyl]methoxybenzoic acid (CAS: 948301-30-2) were synthesized separately using a modified Hegyes method [58] from sodium 4-(methoxycarbonyl)phenolate (Oxchem Corporation, Wood Dale, IL) and (3-chloropropyl)trimethoxysilane (CAS: 2344-83-4; Gelest, Inc.) and from (chloromethyl)dimethyl-β-butyloxysilane (CAS: 3121-75-3; Jihan Pharmaceutical Technology Co., Ltd., Jinan City, Shandong, China) separately (Fig. 1). The N-protected amino acid “B” components were coupled separately to the “C” component amine derivatives 4-
amino-2,2,6,6-tetramethylpiperidine or aminomethylenediphosphonate tetraethyl ester in solution using a peptide coupling reagent benzotriazol-1-yl oxytris(dimethylamino)phosphonium hexafluorophosphate (BOP) or benzotriazol-1-yl oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) in the presence of N,N-Diisopropylethylamine (DIEA). The trifluoroacetic acid (TFA) deprotected “B-C” amino acid amide derivatives were acylated with the organosilicon “A” components in solution using a BOP coupling method to provide the desired organosilicon acyl-amino acid amide small molecules with an “A-B-C” structure (Fig. 1). The compounds were purified by preparative high-performance liquid chromatography.
(HPLC) or crystallization and characterized by analytical HPLC, thin-layer chromatography (TLC) and liquid chromatography-mass spectrometry (LC-MS).

Cell culture and reagents

Human PCa cell lines LNCaP (American Type Culture Collection, Manassas, VA), C4-2, and C4-2B (provided by Dr. Leland WK Chung, Cedars-Sinai Medical Center, Los Angeles, CA) were routinely maintained in T-medium (ThermoFisher Scientific, Waltham, MA) with 5% fetal bovine serum (FBS; Atlanta Biologicals, Atlanta, GA). Docetaxel-resistant C4-2B-TaxR [31] and abiraterone-resistant C4-2B-Abir [59] cells were provided by Dr. Allen C. Gao (University of California Davis, Davis, CA). C4-2B-TaxR cells were routinely maintained in RPMI1640 medium (Corning Inc., Corning, NY) supplemented with 10% FBS and 100 nM docetaxel (LC Laboratories, Woburn, MA). The final concentration of docetaxel in the culture medium was reduced to 5 nM before experimental assays. C4-2B-Abir cells were routinely maintained in the presence of 10 μM abiraterone acetate (Selleck Chemicals, Houston, TX). CWR22Rv1 cells (provided by Dr. Jin-Tang Dong, Emory University, Atlanta, GA) were cultured in RPMI1640 medium supplemented with 10% FBS, 1.5 g/L sodium bicarbonate (Corning), 4.5 g/L glucose, 10 mM Hepes and 1 mM sodium pyruvate (Hyclone, Logan, UT). PC-3 (ATCC) and DU145 (provided by Dr. Geou-Yarh Liou, Clark Atlanta University) were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM, Corning) supplemented with 10% FBS, Cycloheximide (CHX) and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (St. Louis, MO). DMSO was used as the vehicle control in invitro and in vivo studies.

In vitro cytotoxicity assay

In vitro anticancer activity of silicon-containing compounds in the NCI-60 panel of human cancer cell lines was evaluated at the National Cancer Institute Developmental Therapeutics Program (Rockville, MD). Cell viability after 48 h of incubation was determined by sulforhodamine B staining. Growth inhibition of 50% (GI50) was calculated as the drug concentration resulting in a 50% reduction in the net protein increase in control cells during the drug incubation [60]. To determine the half minimal inhibitory concentration (IC50), defined as the concentration of drug required for 50% inhibition of cell proliferation after 72 h of incubation, CellTiter 96 Aqueous non-radioactive cell proliferation assay kit (Promega, Madison, WI) or cell counting kit-8 (CCK-8, Dojindo Molecular Technologies, Inc., Rockville, MD) were used following the manufacturers’ instructions. IC50 values were calculated with SigmaPlot program (Systat Software Inc., San Jose, CA).

Polymerase Chain Reaction (PCR) analysis

Total RNA was prepared with Qiagen RNeasy Kit (Valencia, CA). The first-strand cDNA was synthesized using SuperScript III First-Strand Synthesis System (ThermoFisher Scientific). Quantitative PCR (qPCR) was performed by the Stratagene Mx3005P system (Agilent Technologies, Santa Clara, CA) using a Brilliant SYBR Green QPCR Master Mix (Stratagene, San Diego, CA) according to the manufacturer’s instructions. PCR primers used in this study are listed in Table S1.

Western blot analysis

Total cell lysates were prepared using Radioimmunoprecipitation (RIPA) Lysis and Extraction Buffer (ThermoFisher Scientific). Immunoblotting analyses were performed following standard procedures. Primary antibodies used in this study are listed in Table S2.

Protein half-life determination

PCa cells were incubated with CHX (50 μg/ml) for 2 h to inhibit further protein synthesis and then treated with DMSO or GH1504 for varying times. Total cell lysates were analyzed by Western blotting. The desired protein bands were quantitated and normalized by the intensity of the corresponding loading controls using the ImageJ program, and the data were graphed using the SigmaPlot program. The protein degradation rate was expressed as half-life (T1/2), the time for degradation of 50% of the protein, which was determined by an exponential decay fitting algorithm.

In vivo xenograft tumor growth

All animal procedures performed in this study were approved by Augusta University Institutional Animal Care and Use Committee (IACUC) and followed the National Institutes of Health guidelines. For the subcutaneous model, 2.0 × 10^6 CWR22Rv1 cells were mixed with Matrigel™ Membrane Matrix HC (Corning) and inoculated into both flanks (1.0 × 10^6 cells per site) of male athymic nude mice (Hsd: athymic nude-nu; 5 weeks old; Envigo RMS, Inc, Indianapolis, IN). Tumor-bearing mice were randomly divided into 2 groups and treated with vehicle control (DMSO; 5 mice) or GH1504 at 7.5 mg/kg (8 mice), 3 times per week, via intraperitoneal injection. Mice were weighed twice per week, and tumor sizes were measured and calculated using the formula of (length × width^2 × ½).

For the intratibial xenograft model, a total of 2.0 × 10^6 C4-2B-TaxR cells were inoculated into the bilateral tibiae of male athymic nude mice (5 weeks old). Following the confirmation of tumor formation by rising prostate-specific antigen (PSA) levels in mouse sera (≥ 1.0 ng/ml), mice were randomly divided into 2 groups and treated with vehicle control (6 mice) or GH1504 at 7.5 mg/kg (5 mice), 3 times per week, via intraperitoneal injection. Mice were weighed twice per week, and tumor growth in bilateral tibiae was followed by serum PSA using an enzyme-linked immunosorbent assay (ELISA) kit from United Biotech, Inc (Mountain View, CA). At the end point, X-ray radiography was performed using MX-20 System (Faxitron, Tucson, Arizona). Major organs were examined for any abnormality.

Histopathology

Haematoxylin and eosin (H&E) and immunohistochemistry (IHC) in xenograft tissues were performed following standard procedures. The antibodies used in IHC are listed in Table S2.

Statistical analysis

Each in vitro experiment was repeated independently at least three times. All results were presented as means ± standard error of the mean (SEM). Unpaired, two-tailed, Student t-test was used in statistical analyses. p < 0.05 was taken as a significant difference between means. To assess the longitudinal effect of treatment on tumor growth, a two-way analysis of variance (ANOVA) was performed to test the overall difference across different treatment groups during the entire study period. GraphPad Prism version 8.0 (GraphPad Software Inc., La Jolla, CA) was used to perform the statistical analyses.

Results

Anticancer activity screen of organosilicon compounds in the NCI-60 human cancer cell panel

To obtained an unbiased assessment of newly-developed organosilicon compounds as potential anticancer agents, we submitted five compounds
Table 1

| Name       | Chemical Formula         | Linear Formula | Molecular Weight | CAS Number  |
|------------|--------------------------|----------------|------------------|-------------|
| GH1501     | MSipob-OC2Y-Atmp         | C₃₈H₅₁Cl₂N₃O₂Si | 712.83           | 1648613-37-9|
| GH1502     | MSipob-Bip-Atmp          | C₃₇H₅₃N₃O₂Si   | 613.92           | 1648613-38-0|
| GH1503     | BmSimob-OC2Y-Atmp        | C₂₉H₅₃Cl₂N₃O₂Si| 726.86           | 1648613-40-4|
| GH1504     | BmSimob-Bip-Atmp         | C₂₉H₅₃N₃O₂Si   | 627.94           | 1648613-41-5|
| GH1505     | MSipob-Bip-AMDP(OEt)₄   | C₃₇H₅₄O₉P₂Si   | 760.88           | 1648613-42-6|

Fig. 2. In vitro effects of GH1504 in human cell lines. (A) Growth inhibition of 50% (GI₅₀) of GH1504 in 9 types of human cancer cells in the NCI-60 panel. GI₅₀ was calculated as the drug concentration resulting in a 50% reduction in the net protein increase in control cells after 48 h of incubation. (B) GI₅₀ of GH1504 in all human cell lines included in the NCI-60 panel. (C) Half minimal inhibitory concentration (IC₅₀) of GH1504 in established PCa cells. IC₅₀ was determined as the concentration of drug required for 50% inhibition of cell proliferation after 72 h of incubation.
### Table 2

GI<sub>50</sub> (nM) of selected anticancer small-molecule compounds in NCI-60 panel.

| Panel/Cell Line  | GH1501 | GH1502 | GH1503 | GH1504 | GH1505 |
|------------------|--------|--------|--------|--------|--------|
| **Breast Cancer**|        |        |        |        |        |
| BT-549           | 2010   | 1990   | 2110   | 1960   | 2090   |
| HS 578T          | 1920   | 1670   | 2010   | 1850   | 2610   |
| MCF-7            | 335    | 506    | 356    | 332    | 1880   |
| MDA-MB-231/ATCC  | 881    | 1360   | 494    | 880    | 1620   |
| MDA-MB-468       | 1700   | 1640   | 1540   | 198    | 1720   |
| T-47D            | 1900   | 1920   | 1840   | 1820   | 3100   |
| **Average**      | 1458   | 1514   | 1392   | 1173   | 2170   |
| **Colon Cancer** |        |        |        |        |        |
| COLO 205         | 216    | 392    | 342    | 215    | 1840   |
| HCC-2998         | 1050   | 1560   | 1070   | 1110   | 2020   |
| HCT-15           | 357    | 437    | 323    | 309    | 2250   |
| HCT-116          | 206    | 297    | 319    | 218    | 1930   |
| HT29             | 263    | 430    | 342    | 315    | 2020   |
| KM12             | 363    | 413    | 382    | 376    | 2610   |
| SW-620           | 313    | 392    | 318    | 316    | 2190   |
| **Average**      | 395    | 560    | 442    | 408    | 2123   |
| **CNS Cancer**   |        |        |        |        |        |
| SF-268           | 903    | 1200   | 611    | 679    | 2070   |
| SF-295           | ——     | ——     | ——     | ——     | ——     |
| SF-539           | 1770   | 1810   | 1730   | 1770   | 1820   |
| SNB-19           | 1720   | 1690   | 1600   | 1550   | 2060   |
| SNB-75           | 1340   | 1320   | 1350   | 1630   | ——     |
| U-251            | 230    | 443    | 234    | 238    | 2090   |
| **Average**      | 1193   | 1347   | 1105   | 1173   | 2010   |
| **Leukemia**     |        |        |        |        |        |
| CCRF-CEM         | 1200   | 1380   | 531    | 1500   | 2780   |
| HL-60(TB)        | 813    | 1150   | 790    | 1220   | 2940   |
| K-562            | 322    | 416    | 333    | 386    | 3460   |
| MOLT-4           | 353    | 744    | 315    | 775    | 2860   |
| RPMI-8226        | 1320   | 1800   | 893    | 1950   | 1970   |
| SR               | ——     | ——     | ——     | ——     | ——     |
| **Average**      | 802    | 1098   | 572    | 1166   | 2802   |
| **Melanoma**     |        |        |        |        |        |
| LOX IMVI         | 197    | 212    | 191    | 207    | 1850   |
| M14              | 341    | 466    | 334    | 286    | 2100   |
| MALME-3M         | 1460   | 1610   | 1200   | 1750   | 1930   |
| MDA-MB-435       | 269    | 305    | 250    | 292    | 1900   |
| SK-MEL-2         | 1770   | 2050   | 1560   | 944    | 2110   |
| SK-MEL-5         | 1720   | 1740   | 1680   | 234    | 1580   |
| SK-MEL-28        | 1500   | 1690   | 1580   | 1330   | 1760   |
| UACC-62          | 1490   | 1720   | 1500   | 1150   | 1620   |
| UACC-257         | 1880   | 1840   | 1870   | 1780   | 2420   |
| **Average**      | 1181   | 10047  | 1129   | 886    | 1919   |
| **Non-Small Cell Lung Cancer** | | | | | |
| A549/ATCC        | 407    | 1140   | 332    | 326    | 2840   |
| EKVX             | ——     | ——     | ——     | ——     | ——     |
| HOP-62           | 1860   | 1820   | 1510   | 1360   | 2009   |
| HOP-92           | 1170   | 1280   | 1110   | 1370   | 2090   |
| NCI-H23          | 1650   | 1830   | 1560   | 1760   | 3050   |
| NCI-H226         | 1770   | 1770   | 1340   | 183    | 1840   |
| NCI-H322M        | 1740   | 1840   | 1570   | 1710   | 5110   |
| NCI-H460         | 253    | 370    | 259    | 348    | 1970   |
| NCI-H522         | 1820   | 1940   | 1970   | 1060   | 2020   |
| **Average**      | 1334   | 1499   | 1206   | 1015   | 2615   |

(continued on next page)
GH1504 promotes protein degradation of AR, AR-v7 and survivin in CWR22Rv1 cells

We identified several downstream targets of GH1504 that may mediate its effects in ADT-resistant CWR22Rv1 cells. As shown in Fig. 3A, GH1504 treatment reduced the expression of AR, AR-v7 and survivin in a time-dependent manner. Heat-shock protein 27 (HSP27), an oncogenic protein that may play an important role in CRPC progression and therapeutic resistance [64,65], was also downregulated at 72 h following GH1504 incubation. On the other hand, expression of heat-shock protein 90 (HSP90), a molecular chaperon of AR and survivin [66–68], was not affected. We further investigated the mechanism by which GH1504 inhibited the expression of AR, AR-v7 and survivin. qPCR analyses found that GH1504 only slightly increased AR-v7 expression at 24 h but did not significantly change the RNA levels of AR and survivin during the time course between 0 h and 24 h (Fig. 3B). To determine whether GH1504 affected the stability of these proteins, a CHX pulse-chase assay was performed. In the presence of CHX, an inhibitor of de novo protein synthesis, GH1504 treatment caused a significant reduction in the protein levels of AR, AR-v7 and survivin and shortened their predicted T1/2; from 21.8 h to 2.6 h (AR), 13.0 h to 12.2 h (AR-v7) and > 24.0 h to 23.7 h (survivin), respectively (Fig. 3C). These results indicated that GH1504 may promote protein degradation of AR, AR-v7 and survivin in CWR22Rv1 cells.

GH1504 inhibits the in vivo growth of CWR22Rv1 tumors in mice

We determined the efficacy of GH1504 against the in vivo growth of ADT-resistant PCa. CWR22Rv1 tumors were inoculated subcutaneously into male athymic nude mice and treated with vehicle or GH1504 (7.5 mg/kg), respectively, three times per week, via the intraperitoneal route. At the endpoint, the average tumor size of each treatment group was determined as 1096.11 ± 182.45 mm³ (vehicle) and 866.55 ± 221.72 mm³ (GH1504), respectively. Statistical analyses showed that compared with the vehicle control, GH1504 effectively suppressed the subcutaneous growth of CWR22Rv1 tumors (p = 0.014) (Fig. 4A). The treatments with both vehicle.

Table 2 (continued)

| Panel/Cell Line | GH1501 | GH1502 | GH1503 | GH1504 | GH1505 |
|-----------------|--------|--------|--------|--------|--------|
| IGROV1          | 1200   | 1540   | 704    | 1640   | 3222   |
| NCI/ADR-RES     | 1480   | 1710   | 1250   | 1540   | 3310   |
| OVCAR-3         | 1250   | 1780   | 761    | 202    | 1870   |
| OVCAR-4         | 1620   | 1720   | 711    | 521    | 2540   |
| OVCAR-5         | 1680   | 1870   | 1620   | 1810   | 1710   |
| OVCAR-8         | 268    | 662    | 263    | 216    | 2960   |
| SK-OV-3         | 2080   | 2110   | 2030   | 2120   | 2590   |
| Average         | 1368   | 1627   | 1048   | 1150   | 2600   |
| Prostate Cancer |        |        |        |        |        |
| DU145           | 347    | 541    | 274    | 368    | 1930   |
| PC-3            | 1320   | 1530   | 480    | 1300   | 2100   |
| Average         | 834    | 1036   | 382    | 834    | 2015   |
| Renal Cancer    |        |        |        |        |        |
| 786-0           | 369    | 1490   | 468    | 780    | 2760   |
| A-49B           | 1750   | 1770   | 1950   | 1950   | 3640   |
| ACHN            | 1320   | 1440   | 478    | 402    | 2270   |
| CANK-1          | 1360   | 1620   | 1080   | 1580   | 2900   |
| RXF 393         | 1510   | 1640   | 392    | 179    | 1870   |
| SN12C           | 564    | 1490   | 613    | 526    | 1710   |
| TK-10           | 1850   | 1920   | 1890   | 1110   | 2500   |
| UO-31           | 1520   | 1610   | 1500   | 1550   | 2770   |
| Average         | 1280   | 1623   | 1046   | 1010   | 2553   |
| 60 Cell Lines Average | 1128 | 1316 | 968    | 973    | 2335   |
Fig. 3. In vitro Effects of GH1504 on the expression of AR, AR-v7 and survivin in CWR22Rv1 cells. (A) Protein expression of AR, AR-v7 and survivin in CWR22Rv1 cells treated with 0.9 μM GH1504 at the indicated time points. (B) qPCR analyses of RNA expression of AR, AR-v7 and survivin in CWR22Rv1 cells treated with 0.9 μM GH1504 at the indicated time points. *p > 0.05 for all pairwise comparisons between 0 h and the indicated time points for all genes, except for that between 0 h and 24 h of AR-v7 (*p = 0.035). (C) CWR22Rv1 cells were pre-incubated with CHX (50 μg/ml, 2 h) prior to treatment with DMSO or GH1504 (0.9 μM) at the indicated time points. Protein expression of AR, AR-v7 and survivin was analyzed by Western blotting and quantitated using the ImageJ program.
and GH1504 were associated with gains in the body weight of animals, specifically 26.69% in the control group and 11.21% in the GH1504 group (Fig. 4B). No significant adverse effects on animal behavior were observed. IHC analyses were performed to determine the in vivo effects of GH1504 on the expression of AR, AR-v7, and survivin in CWR22Rv1 tumor specimens (Fig. 4D). Compared with the control group, GH1504-treated tumors had reduced expression of AR, AR-v7, and survivin at tissue levels. These results demonstrated that as a monotherapy, GH1504 inhibited the in vivo growth of ADT-resistant PCa cells in immunocompromised mice.

**GH1504 promotes protein degradation of AR and survivin in C4-2B-TaxR cells**

GH1504 exhibited a low micromolar IC_{50} (2.38 μM) in the highly docetaxel-resistant C4-2B-TaxR cell [31] (Fig. 2C). Similar to its effect in CWR22Rv1 cells, GH1504 markedly reduced the protein expression of AR and survivin in a time-dependent manner and temporarily reduced HSP27 protein at 48 h (Fig. 3A). qPCR analyses found that following GH1504 treatment, AR mRNA level began to reduce by 7 h and survivin mRNA expression was lower at 12 h and 24 h (Fig. 3B). We further examined whether GH1504 affected the protein stability of AR and survivin in C4-2B-TaxR cells. In the presence of CHX, GH1504 shortened the predicted T_{1/2} of full-length AR (from 30.2 h to 22.0 h) and survivin (from 39.2 h to 29.0 h) (Fig. 3C). These results indicated that in C4-2B-TaxR cells, GH1504 may affect the expression of AR and survivin at both transcriptional and post-translational levels.

**GH1504 retards the skeletal growth of C4-2B-TaxR xenografts in athymic nude mice**

We evaluated the in vivo efficacy of GH1504 in the intrartibial model of C4-2B-TaxR cells, which mimicked the clinicopathology of bone metastatic, chemoresistant PCa [31,63]. Following the confirmation of successful tumor inoculation, athymic nude mice were randomized into two groups and treated with the vehicle control or GH1504 (5 mg/kg), respectively, three times per week, via the intraperitoneal route. Previous studies from other groups and us have shown that serum PSA level is an excellent indicator of PCa growth in mouse skeletons [44,69]. Following an 11-week treatment, the average PSA level of each treatment group was 150.36 ± 61.29 ng/ml (control) and 85.51 ± 57.94 ng/ml (GH1504), respectively (Fig. 6A). Statistical analysis showed there were significant differences between the vehicle control and GH1504 groups (p = 0.02). The average body weight of mice in the control group was increased by 4.20% at the endpoint, and GH1504 treatment resulted in a slight decrease in the average body weight (-0.16%; Fig. 6B). X-ray radiography demonstrated that GH1504 treatment improved the skeletal architecture and reduced both osteolytic and osteoblastic lesions in tumor-bearing bones (Fig. 6C). No significant adverse effects on animal behavior were observed. Tissue expression of AR and survivin was further evaluated by IHC staining in bone xenografts, which showed that GH1504 could reduce
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Fig. 5. *In vitro* Effects of GH1504 on the expression of AR and survivin in C4-2B-TaxR cells. (A) Protein expression of AR and survivin in C4-2B-TaxR cells treated with 2.3 μM GH1504 at the indicated time points. (B) qPCR analyses of RNA expression of AR and survivin in C4-2B-TaxR cells treated with 2.3 μM GH1504 at the indicated time points. * p < 0.05; ** p < 0.01; *** p < 0.001. (C) C4-2B-TaxR cells were pre-incubated with CHX (50 μg/ml, 2 h) prior to treatment with DMSO or GH1504 (2.3 μM) at the indicated time points. Protein expression of AR and survivin was analyzed by Western blotting and quantitated using the ImageJ program.

**Discussion**

The treatment landscape for PCa has been extensively expanded over the last decade [5]. Several recent trials further demonstrated that the combination of docetaxel with standard ADT, or “chemohormonal therapy,” significantly improved overall survival in patients with hormone-sensitive PCa [6,7]. Unfortunately, many patients develop extreme drug resistance and have a poor prognosis. There is a great need for novel agents that can overcome resistance to both ADT and docetaxel chemotherapy. Given the central role of AR in CRPC progression, recent efforts have heavily been focused on developing more specific and potent inhibitors of AR signaling, and several new antiandrogens have entered clinical phases [14]. Darolutamide, a novel AR antagonist targeting both wild-type and mutated LBD variants, received FDA approval in 2019 for the treatment of CRPC [70]. In comparison, no significant progress has been made in developing a treatment to overcome chemoresistance. Since 2010, there has been only one registered trial in docetaxel-resistant CRPC, and that trial failed to demonstrate any survival benefits of figitumumab, a monoclonal antibody against insulin-like growth factor-1 receptor (IGF-1R) [71].

Using a combination of rational drug design, phenotypic screening and target deconvolution approaches, we have developed several generations of anticancer compounds. These small molecules share a common structure...
consisting of three pharmacophores derived from approved drugs, natural compounds and chemical moieties with known biological functions [39,42–46,48]. Recently, we reported two small-molecule compounds as potential leads against drug-resistant PCa. Specifically, an aminobisphosphonate-containing compound BKM1972 effectively inhibited the skeletal growth of chemoresistant C4-2B-TaxR cells [44], and an α-phenylcyanic acid derivative LG1836 significantly extended the survival of mice carrying ADT-resistant CWR22Rv1 tumors [48]. Although these results are promising, the anticancer potency of the two compounds remained suboptimal. For example, the IC50 values of BKM1972 in CWR22Rv1 and C4-2B-TaxR cells were 8.4 μM and 4.9 μM, and the IC50 of LG1836 in the two cell lines were 2.6 μM and 4.0 μM, respectively. To discover more effective candidates with the goal of targeting drug-resistant PCa, we conducted C/Si-exchange modifications to our 3-component chemicals and developed a panel of silicon-containing compounds. As we presented here, GH1504 had much improved cytotoxicity in these cells, specifically, with an IC50 of 0.89 μM in CWR22Rv1 and 2.89 μM in C4-2B-TaxR cells. Animal experiments further demonstrated that GH1504 alone was effective against the in vivo growth of CWR22Rv1 and C4-2B-TaxR xenografts. These studies indicated that GH1504 could be further evaluated as a potential lead for overcoming both ADT resistance and chemoresistance in advanced PCa.

Current drug discovery strategies can be largely categorized as target-based drug discovery and phenotypic drug discovery [72]. With the realization of a complex biology and high heterogeneity of human cancers, recent years have seen a renewed interest in the phenotypic approach that has the advantages of identifying first-in-class medicines with novel mechanisms of action. We have followed this route of drug discovery and conducted phenotypic screens of our compounds in-house and at the National Cancer Institute, as in the cases of GH1504 and several other potential leads [44]. We have made efforts to understand the mechanism of action of GH1504 in PCa cells. As described here, GH1504 was found to be capable of co-targeting AR, AR-v7 and survivin, which may collectively contribute to its anticancer activity in drug-resistant PCa cells. Furthermore, an important and remaining question is how GH1504 exerts its inhibitory effect in such a diverse array of human cancer cell lines, including both AR-positive and -negative cells (Fig. 2). While our current study identified several possible targets of GH1504 in these cells, such as HSP27, HSP90, and survivin (Figs. 3A, 5A, S1), it appeared that GH1504 may differentially affect these proteins in a cell context-dependent manner. For example, GH1504 effectively decreases HSP27 but does not alter HSP90 levels in CWR22Rv1 cells, whereas it does not reduce the protein levels of either HSP27 and HSP90 in C4-2B-TaxR cells. These results suggested that HSP27 and HSP90 may be controlled by different mechanisms in CWR22Rv1 and C4-2B-TaxR cells, and the effect of GH1504 on these proteins is a combined result of the compound in these cells. These observations are consistent with previous ones that HSP27 and HSP90 could be regulated at multiple levels by various factors [73,74].

We are in the process of identifying direct molecular target(s) of GH1504. These efforts could provide an explanation for the relatively high potency of GH1504 in a wide spectrum of cancer cell lines and a better understanding of different effects of GH1504 on downstream targets in heterogeneous human cancer cells.

Compared with conventional inhibitors of protein activities, such as enzalutamide and other antiandrogens, inhibition of protein expression may
provide a more effective approach to block oncogenic signaling and improve therapeutic efficacy. Several strategies are being explored to harness the cancer cell’s proteasomal machinery and achieve targeted degradation of oncogenic proteins, including those once considered as undruggable [75]. ARV-110, an orally bioavailable proteolysis-targeting chimera (PROTAC) degrader of AR, is being tested in heavily pretreated metastatic CRPC patients and has demonstrated a favorable preliminary efficacy [76]. Interestingly, our results showed that GH1504 can promote the protein turnover and degradation of AR, AR-V7 and survivin, suggesting that this compound may function as a “co-degrader” of these oncogenic factors. Given the important role of AR, AR-V7 and survivin in ADT resistance and chemoresistance, it is plausible to hypothesize that the simultaneous downregulation of these proteins by GH1504 could elicit higher anticancer activity against drug-resistant CRPC than current compounds targeting a single protein (such as AR-V110).

In conclusion, we have developed a novel anticancer compound GH1504 that effectively inhibited the in vitro and in vivo growth of drug-resistant PCa cells. This is the first silicon-containing small molecule that has been evaluated in preclinical models of PCa. GH1504 and its analogues may represent a new class of drug candidates to overcome therapeutic resistance in PCa patients.

Declaration of Competing Interest
The authors declare that there is no conflict of interest.

CRediT authorship contribution statement
Rui Zhao: Formal analysis. Xiaowei Ma: Formal analysis. Lijuan Bai: Formal analysis. Xin Li: Formal analysis. Kenza Mamouni: Formal analysis. Yang Yang: Formal analysis. Hongyan Liu: Conceptualization. Alira Danaher: Formal analysis. Nicholas Cook: Formal analysis. Omer Kucuk: Conceptualization. Robert S. Hodges: Conceptualization. Lajos Gera: Conceptualization, Formal analysis, Writing – original draft. Daqing Wu: Conceptualization, Writing – original draft.

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Availability of data and materials
All data generated or analyzed during this study are included in this published article.

Ethics approval and consent to participate
Not applicable.

Patient consent for publication
Not applicable.

Supplementary materials
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