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Original Research

Overcoming prostate cancer drug resistance with a novel organosilicon small molecule

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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 intraosseous 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; $AMDP(OEt)_4$, 1-aminomethylenedisphosphonic acid tetraethyl ester amide residue; AR, androgen receptor; AR-V7, AR variant 7; Atmp, 4-amino-2,2,6,6-tetramethylpiperidine amide residue; BmSimob, 4-[(butyldimethylsilyl)methoxy]-benzoyl; Bip, β-(4-biphenylyl)alanine residue; BOP, benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate; COX-2, cyclooxygenase-2; CRPC, castration-resistant prostate cancer; DIEA, N,N-diisopropylethylamine; 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-[3-(trimethylsilyl)propoxy]-benzoyl; NTD, N-terminal domain; OC2Y, O-2,6-dichlorobenzyl-tyrosine residue; PCa, prostate cancer; PROTAC, proteolysis-targeting chimaera; PSA, prostate-specific antigen; PyBOP, benzotriazol-1-yloxytripyrrolidinophophonium hexafluorophosphate; SIAH2, siah E3 ubiquitin protein ligase 2; TFA, trifluoroacetic acid.

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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, apalutamide, 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 liquid 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 oncofetal 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 simultaneous 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 α-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 biologically 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]. Bikzhanova et al. reported that the addition of a silicon to indomethacin, an anti-inflammatory drug, resulted in sila-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 on 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 4-[3-(trimethylsilyl)propoxy]benzoic acid (CAS: 948301-32-4) or 4-[butyl-dimethylsilyl)methoxy]benzoic 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)trimethylsilane (CAS: 2344-83-4; Gelest, Inc.) and from (chloromethyl)dimethyl-*n*-butylsilane (CAS: 3121-75-3; Jiwan 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-

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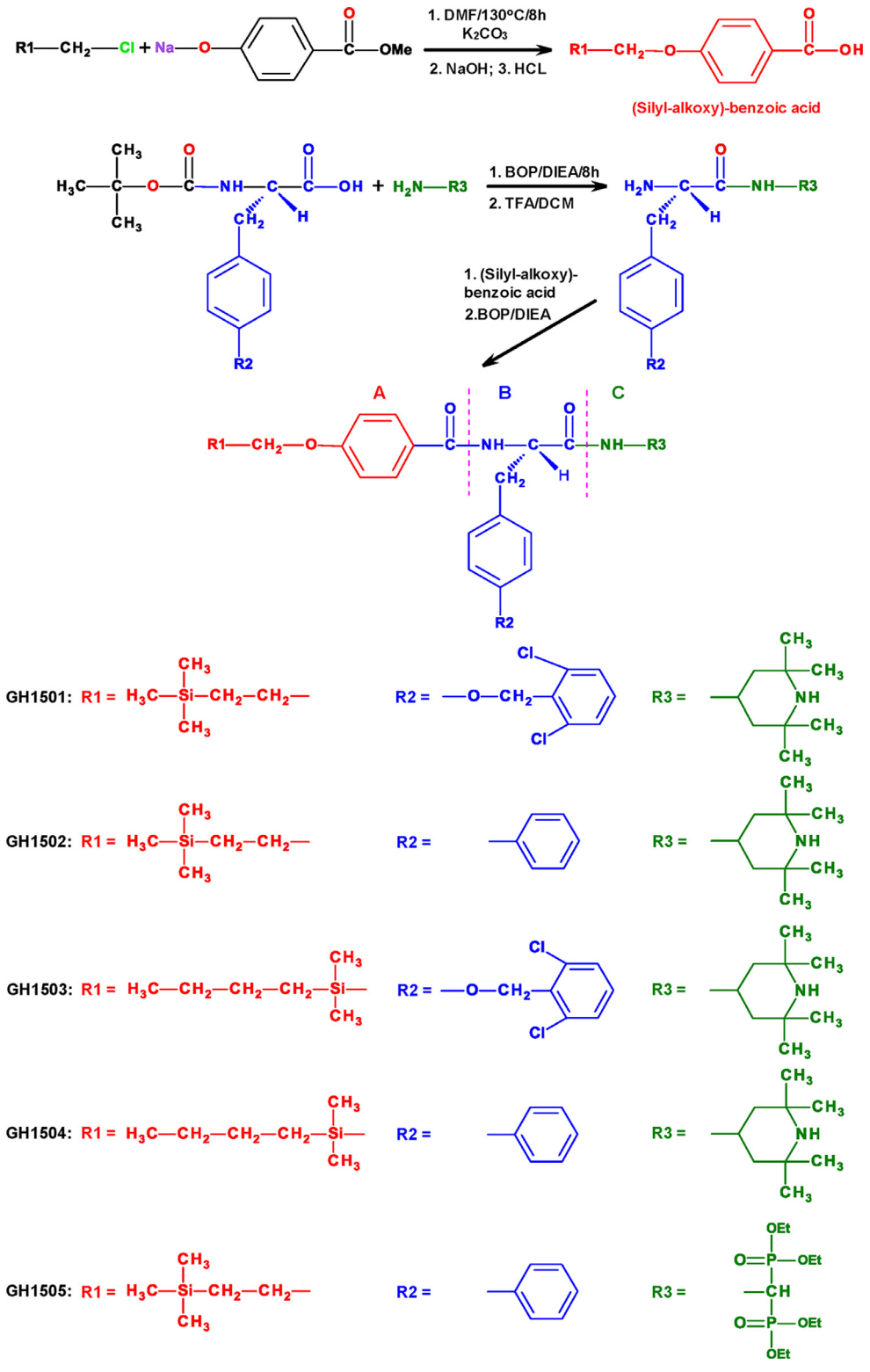


Fig. 1. Synthesis and chemical structures of organosilicon compounds tested in this study. R1 group contains the silicon atom.

amino-2,2,6,6-tetramethylpiperidine or aminomethylenediphosphonate tetraethyl ester in solution using a peptide coupling reagent benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP) or benzotriazol-1-yloxytripyrrolidinophophonium 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 *in vitro* and *in vivo* studies.

In vitro *cytotoxicity assay*

*I*n 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% (GI_{50}) 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 (IC_{50}), 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. IC_{50} 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 ($T_{½}$), 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 $\times$ width^2 x 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 ($\geq$ 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

Haemotoxylin 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 $\pm$ 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

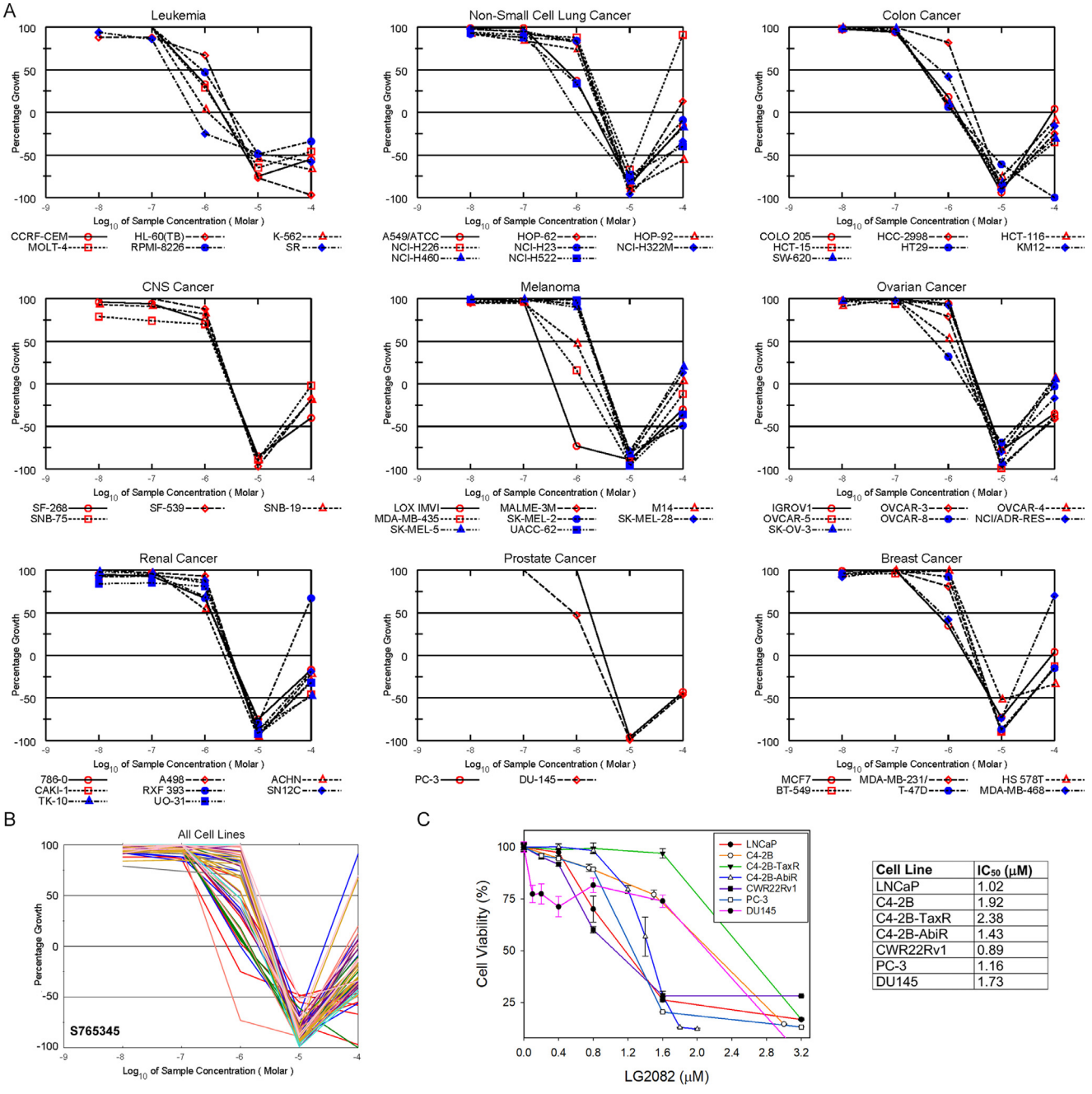
Anticancer activity screen of organosilicon compounds in the NCI-60 human cancer cell panel

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

Table 1

Several novel silicon-containing small-molecule compounds.

Name	Chemical Formula	Liner Formula	Molecular Weight	CAS Number
GH1501	MSipob-OC2Y-Atmp	$C_{38}H_{51}Cl_2N_3O_4Si$	712.83	1648613-37-9
GH1502	MSipob-Bip-Atmp	$C_{37}H_{51}N_3O_3Si$	613.92	1648613-38-0
GH1503	BmSimob-OC2Y-Atmp	$C_{39}H_{53}Cl_2N_3O_4Si$	726.86	1648613-40-4
GH1504	BmSimob-Bip-Atmp	$C_{38}H_{53}N_3O_3Si$	627.94	1648613-41-5
GH1505	MSipob-Bip-AMDP(OEt)$_4$	$C_{37}H_{54}N_2O_9P_2Si$	760.88	1648613-42-6



Cell Line	IC_{50} (μM)
LNCaP	1.02
C4-2B	1.92
C4-2B-TaxR	2.38
C4-2B-AbiR	1.43
CWR22Rv1	0.89
PC-3	1.16
DU145	1.73

Fig. 2. ***In vitro*** **effects of GH1504 in human cell lines.** (A) Growth inhibition of 50% (GI_{50}) of GH1504 in 9 types of human cancer cells in the NCI-60 panel. GI_{50} 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) GI_{50} of GH1504 in all human cell lines included in the NCI-60 panel. (C) Half minimal inhibitory concentration (IC_{50}) of GH1504 in established PCa cells. IC_{50} was determined as the concentration of drug required for 50% inhibition of cell proliferation after 72 h of incubation.

Table 2

GI_{50} (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	2000
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**
Ovarian Cancer					

(*continued on next page*)

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	490	1300	2100
Average	**834**	**1036**	**382**	**834**	**2015**
Renal Cancer					
786-0	369	1490	468	780	2760
A-498	1750	1770	1950	1950	3640
ACHN	1320	1440	478	402	2270
CAKI-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**

(GH1501, GH1502, GH1503, GH1504, and GH1505; Table 1) to the National Cancer Institute Developmental Therapeutics Program for evaluation of their cytotoxic activity in the NCI-60 cell line panel, which contains nine types of diverse cancer cells representing leukemia, melanoma, non-small-cell lung, colon, kidney, ovarian, breast, prostate, and central nervous system cancers [60]. As summarized in Table 2, the five organosilicon compounds effectively suppressed the *in vitro* proliferation of multiple cancer cell lines. Among the tested compounds, GH1503 and GH1504 demonstrated high potency against all NCI-60 cancer cell lines, with an average GI_{50} value of 0.968 μM and 0.973 μM (Fig. 2A, 2B), respectively. Based on the NCI-60 screen results, GH1503 and GH1504 were selected as potential leads for further evaluation.

GH1504 exhibits potent in vitro *cytotoxicity against ADT-resistant and chemoresistant PCa cells*

Firstly, we determined and compared the *in vitro* cytotoxicity of GH1503 and GH1504 in CWR22Rv1 and C4-2B-TaxR, two representative drug-resistant PCa cell lines. CWR22Rv1 cells express both full-length AR (H875Y) and AR-v7, and are resistant to treatment with abiraterone and enzalutamide [61,62]; C4-2B-TaxR is a highly docetaxel-resistant C4-2B derivative that represents the phenotypes of acquired chemoresistance [31]. The IC_{50} values of GH1504 in CWR22Rv1 (0.89 μM) and C4-2B-TaxR (2.38 μM) were found to be lower than those of GH1503 (0.90 μM and 3.29 μM), respectively. Therefore, GH1504 was selected as a potential lead compound for targeting both ADT-resistant and chemoresistant PCa cells. The *in vitro* cytotoxicity of GH1504 was further evaluated in a panel of established PCa cell lines with different molecular and cellular characteristics, including androgen-dependent LNCaP, androgen-independent C4-2 and C4-2B [63], abiraterone-resistant C4-2B-AbiR [59], and AR-negative PC-3 and DU145 cells. The average IC_{50} value of GH1504 in these cell lines was determined as 1.50 μM (Fig. 2C). Taken together, these results indicated that GH1504 had low micromolar cytotoxicity in multiple PCa cells with diverse phenotypes, including those refractory to ADT and docetaxel chemotherapy.

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 $T_{1/2}$ 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 mm^3 (vehicle) and 866.55 ± 221.72 mm^3 (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

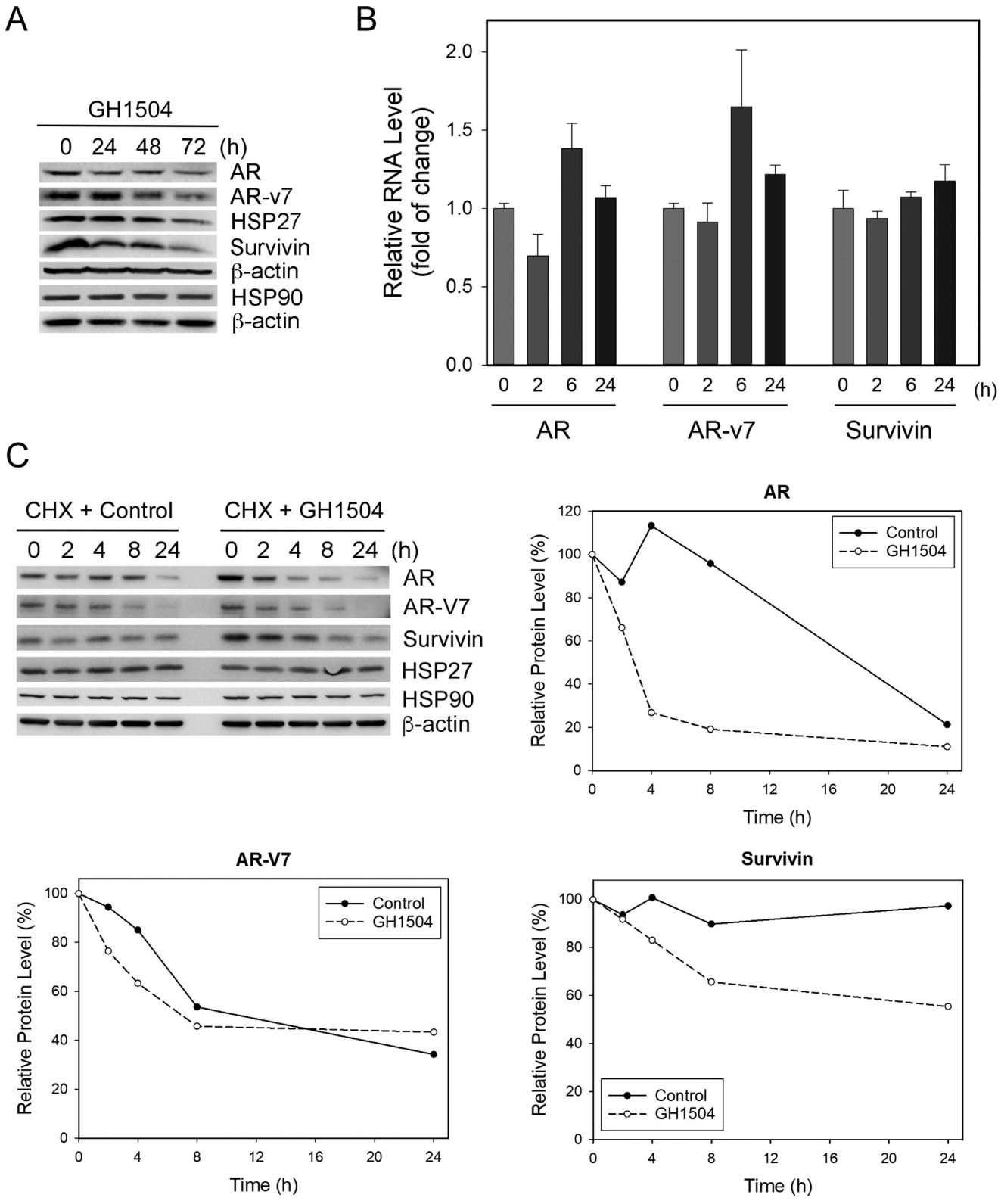


Fig. 3. ***In vitro*** **Effects of GH1504 on the expression of AR, AR-v7 and survivin in CWR22Rv1 cells.** (A) Protein expression of AR, AR-v7 and survivin in CWR22Rv1 cells treated with 0.9 μM GH1504 at the indicated time points. (B) qPCR analyses of RNA expression of AR, AR-v7 and survivin in CWR22Rv1 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.

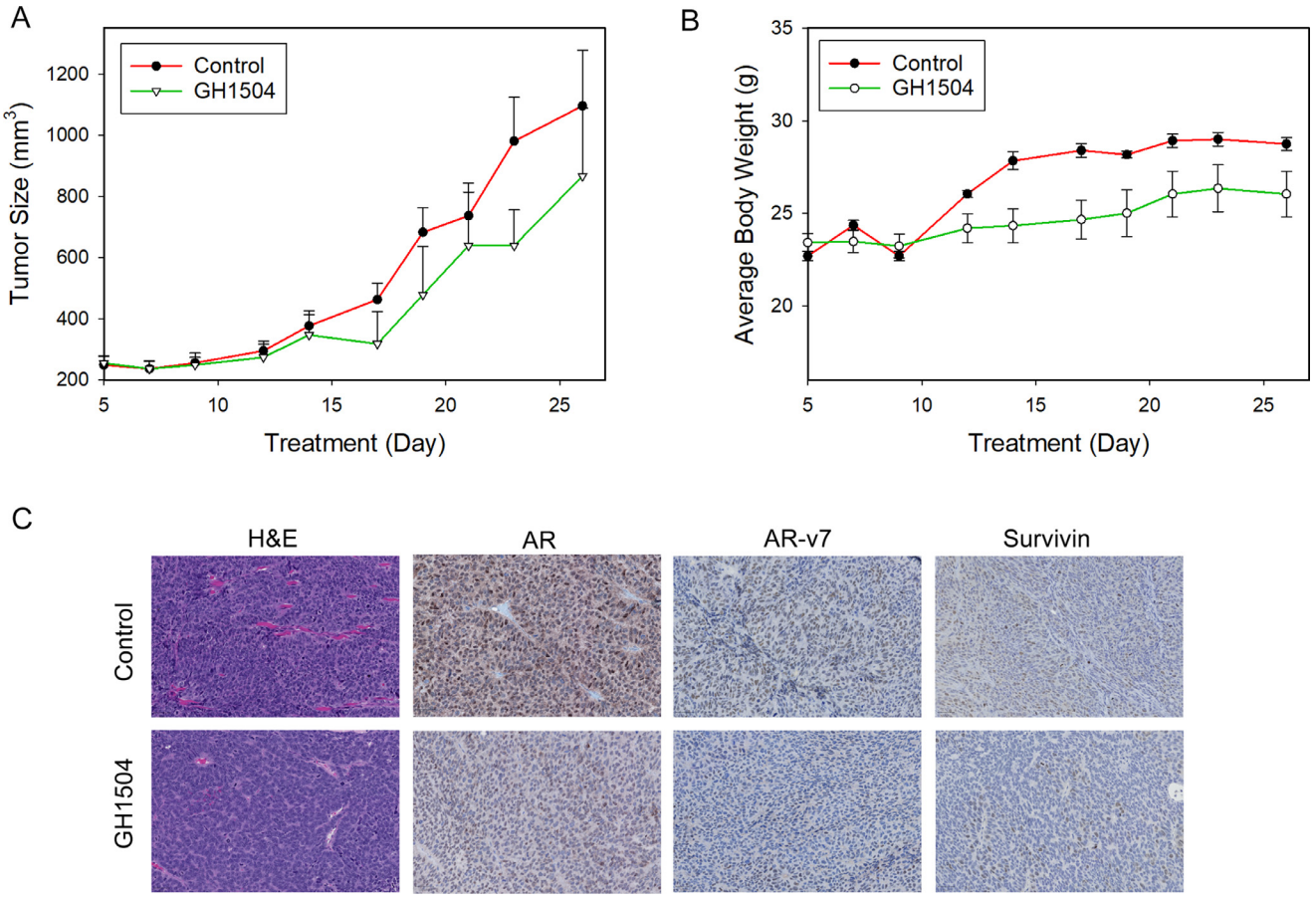


Fig. 4. GH1504 inhibits the subcutaneous growth of CWR22Rv1 tumors in male athymic nude mice. (A) CWR22Rv1 tumor-bearing mice were treated with vehicle ($n = 5$) or GH1504 (7.5 mg/kg, intraperitoneally, 3 times per week; $n = 8$). Two-way ANOVA analysis was used to evaluate the statistical difference of the tumor sizes between different treatment groups. $p = 0.014$. (B) Average body weight of athymic nude mice treated with vehicle or GH1504. (C) H&E staining and IHC expression of AR, AR-v7 and survivin in CWR22Rv1 tumors treated with vehicle or GH1504. Scale bar: 50 μm.

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 cells [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. 5A). qPCR analyses found that following GH1504 treatment, AR mRNA level began to reduce at 6 h and survivin mRNA expression was lower at 12 h and 24 h (Fig. 5B). 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. 5C). 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 intratibial 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 (7.5 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

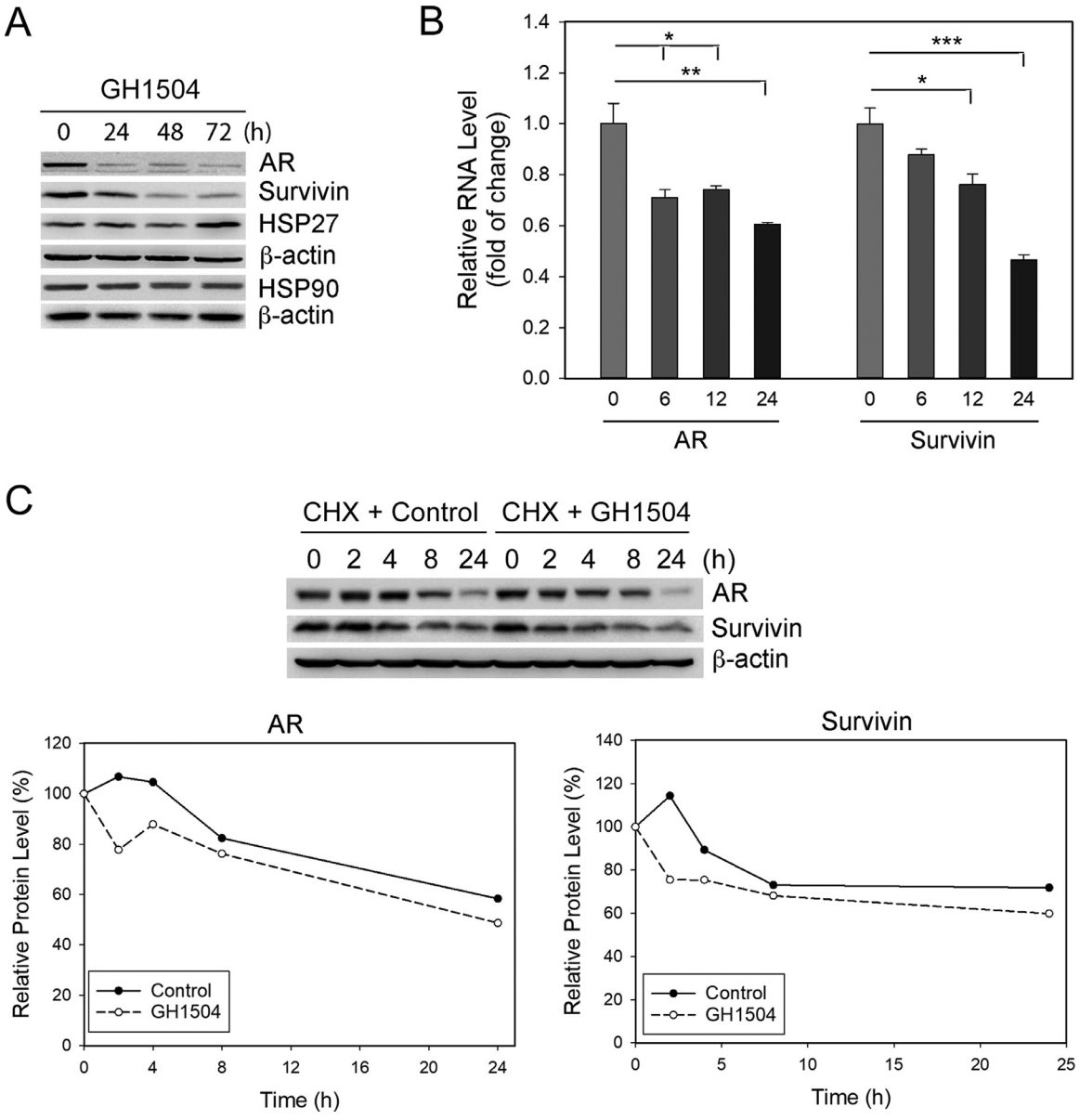


Fig. 5. ***In vitro*** **Effects of GH1504 on the expression of AR and survivin in C4-2B-TaxR cells.** (A) Protein expression of AR and survivin in C4-2B-TaxR cells treated with 2.3 μM GH1504 at the indicated time points. (B) qPCR analyses of RNA expression of AR and survivin in C4-2B-TaxR cells treated 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.

the *in vivo* expression of AR and survivin in C4-2B-TaxR tumors (Fig. 6D). Taken together, these results indicated that GH1504 is effective in retarding the *in vivo* growth of chemoresistant PCa in mouse skeletons.

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

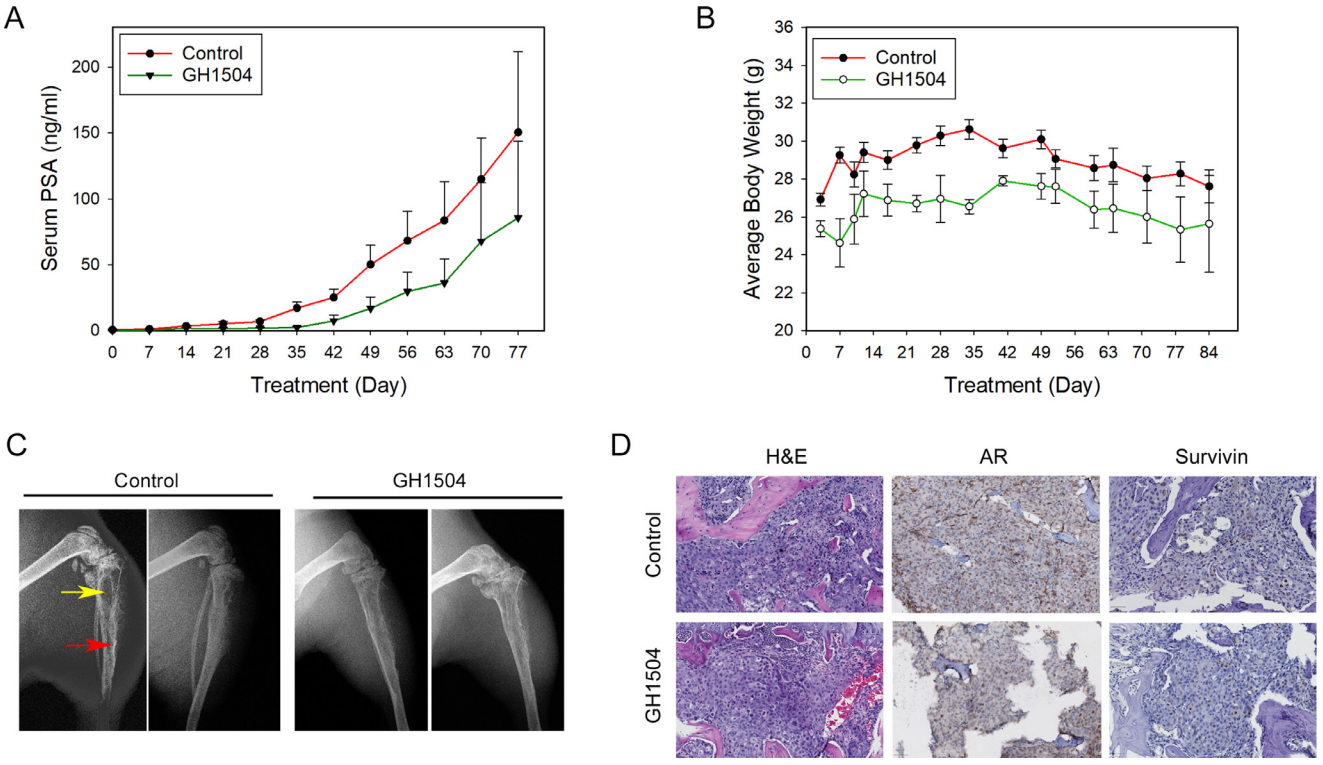


Fig. 6. GH1504 inhibits the intratibial growth of chemoresistant C4-2B-TaxR tumors in male athymic nude mice. (A) Serum PSA values of C4-2B-TaxR tumor-bearing mice treated with vehicle ($n = 6$) or GH1504 (7.5 mg/kg, intraperitoneally, 3 times per week; $n = 5$). Two-way ANOVA analysis was used to evaluate the statistical difference of the PSA values between different treatment groups. $p = 0.02$. (B) Average body weight of athymic nude mice treated with vehicle or GH1504. (C) Representative X-ray radiography of tumor-bearing tibiae in different treatment groups. Yellow arrow: osteolytic lesions; red arrow: osteoblastic lesions. (D) H&E staining and IHC expression of AR and survivin in C4-2B-TaxR bone tumors treated with vehicle or GH1504. Scale bar: 50 μm (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

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-phenylcinnamic 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 IC_{50} values of BKM1972 in CWR22Rv1 and C4-2B-TaxR cells were 8.4 μM and 4.9 μM, and the IC_{50} 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 IC_{50} 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 ARV-110).

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

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.neo.2021.11.006.

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