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MECHANISMS OF RESISTANCE

Susceptibilities of Ugandan *Plasmodium falciparum* Isolates to Proteasome Inhibitors

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ABSTRACT The proteasome is a promising target for antimalarial chemotherapy. We assessed *ex vivo* susceptibilities of fresh *Plasmodium falciparum* isolates from eastern Uganda to seven proteasome inhibitors: two asparagine ethylenediamines, two macrocyclic peptides, and three peptide boronates; five had median IC_{50} values <100 nM. TDI8304, a macrocylic peptide lead compound with drug-like properties, had a median IC_{50} of 16 nM. Sequencing genes encoding the $\beta2$ and $\beta5$ catalytic proteasome subunits, the predicted targets of the inhibitors, and five additional proteasome subunits, identified two mutations in $\beta2$ (I204T, S214F), three mutations in $\beta5$ (V2I, A142S, D150E), and three mutations in other subunits. The $\beta2$ S214F mutation was associated with decreased susceptibility to two peptide boronates, with IC_{50}s of 181 nM and 2635 nM against mutant versus 62 nM and 477 nM against wild type parasites for MMV1579506 and MMV1794229, respectively, although significance could not be formally assessed due to the small number of mutant parasites with available data. The other $\beta2$ and $\beta5$ mutations and mutations in other subunits were not associated with susceptibility to tested compounds. Against culture-adapted Ugandan isolates, two asparagine ethylenediamines and the peptide proteasome inhibitors WLW-vinyl sulfone and WLL-vinyl sulfone (which were not studied *ex vivo*) demonstrated low nM activity, without decreased activity against $\beta2$ S214F mutant parasites. Overall, proteasome inhibitors had potent activity against *P. falciparum* isolates circulating in Uganda, and genetic variation in proteasome targets was uncommon.

KEYWORDS *Plasmodium falciparum*, antimalarial agents, proteasome

Malaria, particularly disease caused by *Plasmodium falciparum*, remains a huge problem, and its control is challenged by resistance to most available therapies (1). New antimalarial drugs, ideally directed against new targets, are needed. One potential target is the proteasome, a protein degradation complex of eukaryotes and actinomycetes that degrades proteins targeted for removal after tagging with ubiquitin or other protein tags (2). A proteasome complex is composed of multi-subunit structures, generally a 20S core particle and one or two 19S regulatory particles on one or both ends of the 20S barrel (2). The 19S regulatory particle recognizes ubiquitinated proteins and transfers them to the 20S core particle for degradation (2). The proteasome has been validated as a target for drugs to treat multiple myeloma and mantle cell lymphoma, and three proteasome inhibitors, bortezomib,

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carfilzomib, and ixazomib, are approved for these indications (3). Additionally, the proteasomes of pathogens have been identified as potential targets for drugs against a number of infectious diseases, including those caused by bacteria and protozoan parasites (4).

In eukaryotes, the 20S core particle contains 14 α and 14 β subunits arranged in a $\alpha7\beta7\beta7\alpha7$ barrel-shaped complex, with 7 different α subunits, including the α-type-6 subunit, forming the outer ring, and 7 different β subunits forming the inner rings. Outer rings regulate the entrance of protein substrates, and proteolytic activity exists in the β rings (5). Only $\beta1$, $\beta2$, and $\beta5$ are catalytically active, with caspase-like, trypsin-like, and chymotrypsin-like proteolytic activities, respectively (6), and the four noncatalytic subunits ($\beta3$, $\beta4$, $\beta6$, and $\beta7$) function in assembly and provide structural support for the proteasome complex (7). The RPN10 subunit in the 19S regulatory particle is an essential canonical ubiquitin receptor that recognizes multiubiquitin chains (8). The C-terminal ubiquitin interacting motif of this subunit identifies ubiquinated substrates to shuttle to the proteasome (2, 5). The 19S regulatory particle contains a 19S PfRPT4 subunit, which is an ATPase located at the base of the 19S particle that interacts with the α-ring of the core particle (5). PF3D7_0808300 encodes a putative *P. falciparum* ubiquitin regulatory protein (9); increased copy number was associated *in vitro* with low grade resistance to WLL-vinyl sulfone (WLL-VS) and WLW-VS (10).

The *P. falciparum* proteasome is similar to that of other eukaryotes, with moderate homology between human and *P. falciparum* catalytic subunits (27% identity for $\beta1$, 53% for $\beta2$, and 51% for $\beta5$; [11]), but differences in structure and substrate specificity have facilitated the design of *P. falciparum*-targeted inhibitors. Among proteasome inhibitors that have been studied for the treatment of malaria are asparagine ethylenediamines (12), peptide vinyl sulfones (13), and peptide boronates (14, 15). Proteasome inhibitors are typically selective for a particular catalytic subunit, but some can act against multiple subunits (12, 16). Asparagine ethylenediamines are noncovalent, reversible inhibitors that specifically target the $\beta5$ subunit (12). Peptide boronates are covalent and slowly reversible inhibitors, and a validated class, with two FDA approved drugs, bortezomib and ixazomib (3, 15). Peptide vinyl sulfones are covalent and irreversible inhibitors, including WLL-VS, which targets the $\beta2$ and $\beta5$ subunits, and WLW-VS, which targets the $\beta2$ subunit (17). Peptide epoxyketones are another validated class of proteasome inhibitors, with one FDA-approved drug (18). Compounds from all of these classes have been developed to selectively target the *P. falciparum* proteasome over its human counterpart (17, 19, 20).

In considering the *P. falciparum* proteasome as a target for antimalarials, it is important to characterize naturally occurring variation in inhibitor susceptibility among circulating parasites. We therefore measured *ex vivo* susceptibilities of fresh Ugandan isolates to a range of proteasome inhibitors. We also sequenced target *P. falciparum* proteasome subunits and explored associations between identified genetic polymorphisms and inhibitor susceptibilities of Ugandan parasites.

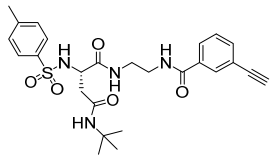
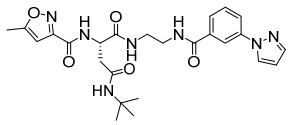
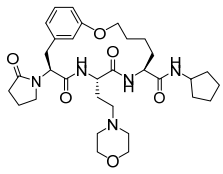
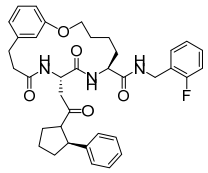
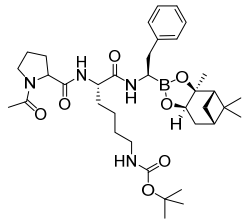
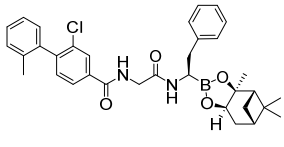
RESULTS

***Ex vivo* susceptibilities of Ugandan *P. falciparum* isolates to proteasome inhibitors.** We characterized the *ex vivo* susceptibilities of fresh Ugandan isolates to 7 proteasome inhibitors; 29 to 234 different isolates were studied per inhibitor, with differences in the number of isolates studied due to varied availability of different inhibitors over time (Table 1). Five of the compounds had median IC_{50} values $<$100 nM (Fig. 1).

We assessed correlations between potencies of tested compounds to gain insight into potential shared mechanisms of action or resistance, but different compounds were studied over time, and correlations were only possible for compounds studied against the same isolates. Spearman coefficients generally showed positive ($r > 0.6$) correlations between compounds of the same chemical class (Fig. 2).

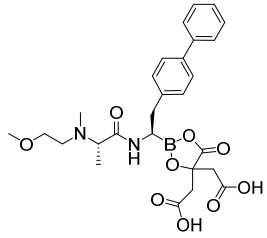
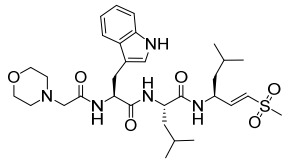
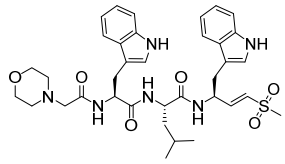
Associations between *P. falciparum* proteasome subunit polymorphisms and *ex vivo* susceptibilities to proteasome inhibitors. We sequenced genes encoding proteasome subunits to assess associations between sequence polymorphisms and drug susceptibility phenotypes (Table 2). The $\beta2$ subunit was sequenced in 726 isolates. Four isolates with $\beta2$ subunit nonsynonymous SNPs were identified; one with a I204T mutation and three with a S214F mutation, each a mixed isolate with both wild type and mutant sequences.

TABLE 1 Proteasome inhibitors studied[a]

Compound	Chemical class	Structure	Isolates studied *ex vivo*	*Ex vivo* IC50 (median, nM)
WZ32 (12, 21, 22)	Asn ethylenediamine		33	7.3
TDI4258 (12, 21, 22)	Asn ethylenediamine		38	28
TDI8239 (25)	Macrocyclic peptide		29	49
TDI8304 (12, 21, 22)	Macrocyclic peptide		195	16
MMV1579506	Peptide boronate		234	58
MMV1581599	Peptide boronate		127	404

(Continued on next page)

TABLE 1 (Continued)

Compound	Chemical class	Structure	Isolates studied *ex vivo*	*Ex vivo* IC50 (median, nM)
MMV1794229 (19)	Peptide boronate		126	206
WLL-VS (17)	Peptide vinyl sulfone		NS	NS
WLW-VS (17)	Peptide vinyl sulfone		NS	NS

[a]NS, not studied against *ex vivo* samples.

Both of these mutations are located in the C-terminal tail of the β2 subunit (Fig. 3). Susceptibilities for three of these isolates were available for MMV1579506, and for two of the isolates for MMV1794229 and TDI8304. The β2 S214F mutation was associated with decreased susceptibility to two peptide boronates (IC_{50} 181 nM and 2635 nM against mutant in all cases mixed isolates) versus 62 nM and 477 nM against wild type parasites for MMV1579506 and MMV1794229, respectively, but not to TDI8304 (IC_{50} 13 nM against mutant versus 17 nM against wild type parasites), although analyses were limited by the small number of mutant parasites available for study (Fig. 3).

The β5 subunit was sequenced in 786 isolates. We identified 4 mixed isolates with a A142S polymorphism, one mixed isolate with a D150E polymorphism, and 10 isolates with a V2I polymorphism (one pure mutant and nine mixed isolates; as this amino acid is not part of the mature β5 subunit after amino-terminal cleavage, these isolates were not further analyzed). One isolate with the A142S polymorphism and one with the D150E polymorphism had IC_{50} data available for TDI8304 and TDI4258; the polymorphisms did not appear to be associated with altered inhibitor susceptibility (Fig. 3).

We also sequenced other *P. falciparum* proteasome components and searched for associations between identified polymorphisms and susceptibility to inhibitors (Table 2). No polymorphisms were identified in the fourth exon of the β6 subunit, which encodes a protein segment adjacent to the β5 subunit in the *P. falciparum* proteasome (123 isolates sequenced), in the 19S PfRPT4 subunit (893 isolates), or in the α-type-6 subunit (893 isolates). Of 833 isolates with the RPN10 subunit sequenced, 213 had a T225S polymorphism and 274 had a E380L polymorphism. These polymorphisms were not associated with altered susceptibility to any of the tested inhibitors. Of 627 isolates with PF3D7_0808300 sequenced, 141 had an M144I polymorphism. This polymorphism was not associated with altered susceptibility to any of the tested inhibitors.

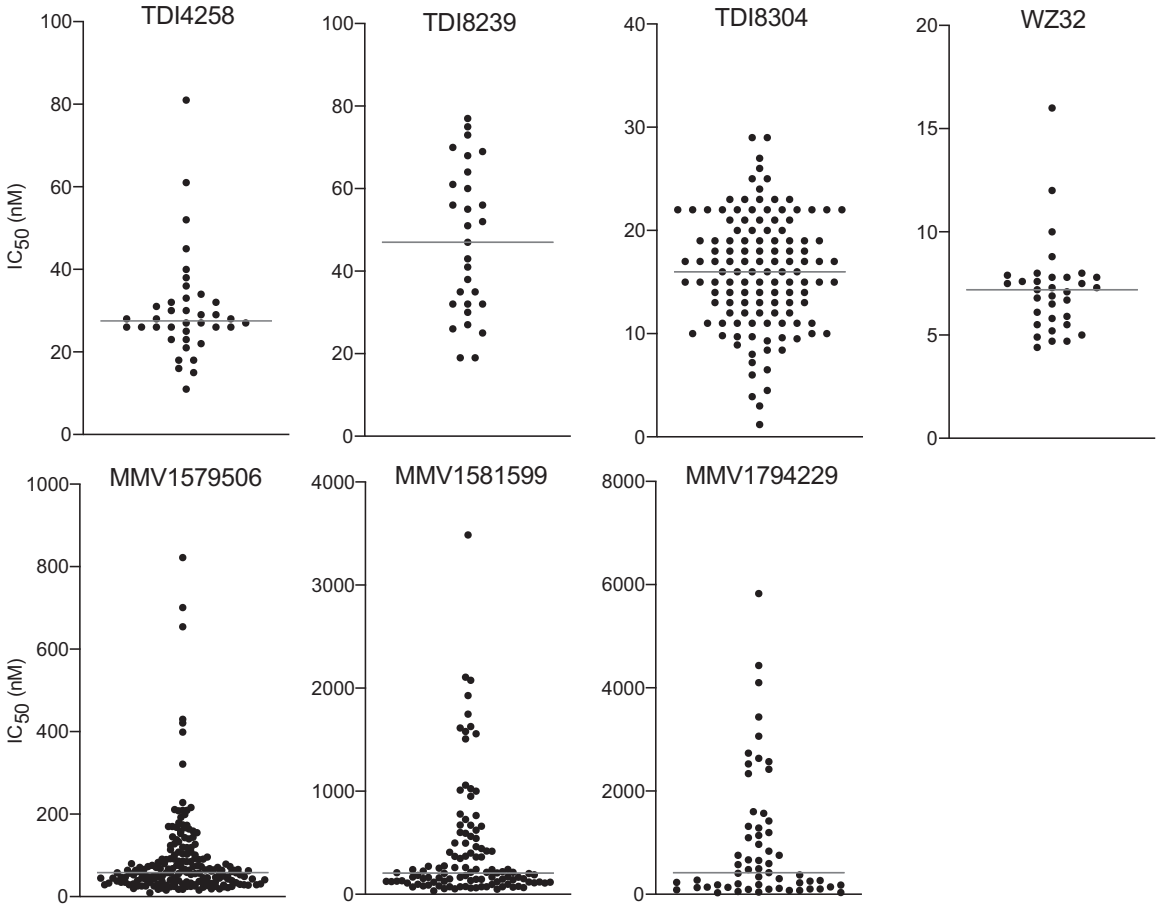


FIG 1 Susceptibilities of Ugandan *P. falciparum* isolates to proteasome inhibitors. Results for each isolate are represented by a dot. Horizontal lines indicate median IC_{50}s.

Susceptibility of culture adapted parasites with the β2 S214F mutation to proteasome inhibitors. To further study the relevance of the β2 S214F mutation, which was associated with decreased susceptibility to MMV1579506 and MMV1794229 in fresh Ugandan isolates, we measured inhibitor susceptibilities of a culture adapted Ugandan clone with the wild type β2 sequence; a culture adapted Ugandan isolate, which was pure β2 214F mutant after culture adaptation; a laboratory clone derived from the V1/S $K13^{C580Y}$ strain that was selected for the β2 49E mutation (10), and strain W2, which has wild type β2 sequence. These studies included TDI4258, TDI8304, MMV1579506, and MMV1794229, and also two peptide vinyl sulfones targeting the β2 (WLW-VS) and $\beta2/\beta5$ (WLL-VS) subunits that were not available for earlier *ex vivo* studies against Ugandan isolates. Susceptibilities to WLW-VS, WLL-VS, TDI4258, TDI8304, MMV1579506 and MMV1794229 did not differ between Ugandan β2 214 wild type and mutant parasites (mean IC_{50} values 90.7, 8.2, 18.5, 14.4, 93.7, and 309 nM against mutant versus 104, 7.8, 19.2, 12.7, 66.2, and 259 nM against wild type, respectively; Fig. 4).

DISCUSSION

We studied susceptibilities of fresh Ugandan *P. falciparum* isolates to a panel of proteasome inhibitors and searched for associations between susceptibilities and genetic polymorphisms in proteasome targets. A number of the inhibitors had potent activity against *P. falciparum* isolates circulating recently in Uganda. A small number of genetic polymorphisms were identified in the proteasome subunits of the Ugandan isolates. The S214F mutation in the β2 subunit was associated with decreased susceptibility to peptide boronates, but not other tested classes of proteasome inhibitors, although the infrequency

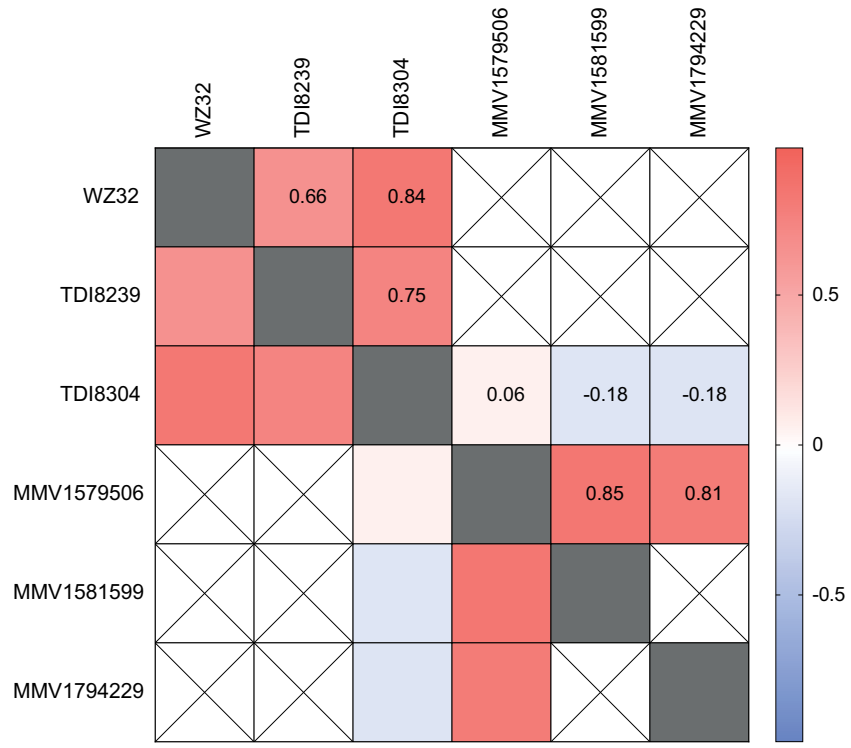


FIG 2 Heat map demonstrating correlations between susceptibilities of Ugandan isolates to different compounds. Spearman's coefficients (r) are indicated numerically and by the color scale. Crossed-out fields represent compounds not studied in parallel, such that correlations could not be measured.

of this mutation limited the analysis. Overall, a number of proteasome inhibitors offered potent activity against *P. falciparum* circulating in Uganda, and genetic variation in the β2 and β5 subunit targets was uncommon.

The 7 tested proteasome inhibitors mostly showed potent *ex vivo* activity, with values similar to those previously seen against cultured *P. falciparum* laboratory strains for asparagine ethylenediamine (12, 21), macrocyclic peptide, and peptide boronate inhibitors (21, 22). Asparagine ethylenediamines and macrocyclic peptides had median IC_{50}s $<$50 nM against the Ugandan isolates, suggesting potential as antimalarial lead compounds. Peptide boronates had varied activity, with median IC_{50} values of 56 to 668 nM against the Ugandan isolates. Among the isolates studied, strong correlations were seen between results for chemically related inhibitors, suggesting shared determinants of activity.

TABLE 2 Mutations identified in proteasome subunits of Ugandan *P. falciparum* isolates

				Median IC_{50} (nM)						
Proteasome subunit	Polymorphism	Isolates sequenced	Isolates with IC_{50} data	WZ32	TDI 4258	TDI 8239	TDI 8304	MMV 1579506	MMV 1581599	MMV 1794229
β2	-	722	25–209	7.0	28	45	17	62	207	477
β2	S214F	3	1–2	-[a]	-	-	13	181	-	2635
β2	I204T	1	1	-	-	-	19	62	-	276
β5	-	781	24–228	7.0	28	35	15	57	145	406
β5	A142S	4	1	-	-	-	17	-	-	-
β5	D150E	1	1	-	28	-	-	-	-	-
β6	-	123	32–123	6.0	27	35	15	57	145	477
19S PfRPT4	-	893	29–232	7.2	28	47	16	58	209	553
α-type-6	-	893	29–232	7.2	28	47	16	58	209	553
RPN10	-	346	12–87	7.3	27	48	16	64	228	569
RPN10	T225S	213	12–60	7.0	28	54	16	61	210	616
RPN10	E380L	274	4–52	7.8	27	41	16	49	163	614
PF3D7_0808300	-	486	16–131	7.2	28	53	15	56	193	668
PF3D7_0808300	M144I	141	4–38	6.9	27	45	17	57	240	401

[a]Dashes indicate that no values were available.

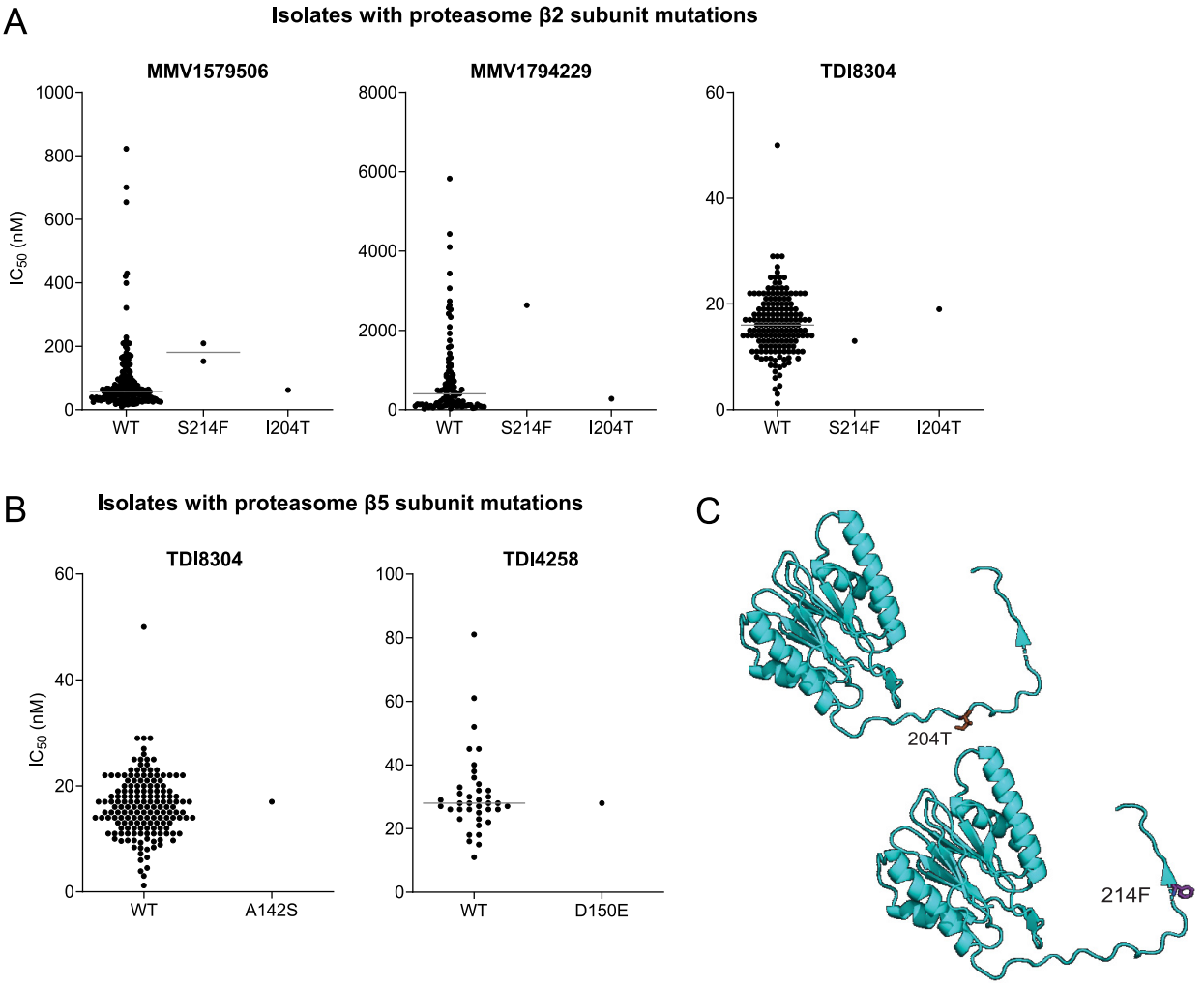


FIG 3 Susceptibilities of mutant *P. falciparum* isolates to proteasome inhibitors. Susceptibilities of isolates with β2 (A) and β5 (B) wild type (WT) or mutant sequences are shown. Results for individual isolates are represented by dots. Horizontal bars indicate median IC_{50} values. C. Structural model of the β2 subunit with the I204T and S214F mutations indicated in purple.

Previous studies showed selection for a number of polymorphisms in different proteasome subunits in *P. falciparum* laboratory strains incubated with proteasome inhibitors (10, 12, 15, 22). To assess genotype variability in circulating Ugandan isolates, we sequenced the β2 and β5 catalytic proteasome subunits, which are predicted inhibitor targets, and 5 regulatory subunits. We identified a small number of polymorphisms in the β2 and β5 subunits, and we searched for associations between these and other polymorphisms and *ex vivo* susceptibilities. The two identified mutations in the β2 subunit, S214F and I204T, were both found in isolates with mixed genotypes. Isolates with one of these mutations, β2 S214F, had decreased susceptibility to two peptide boronates, MMV1579506 and MMV1794229, but the small number of available mutant parasites limited the analysis. The S214F mutation is located in the C-terminal tail of the β2 subunit, which wraps around the β3 subunit (Fig. 3; [23]), but the basis of altered inhibitor sensitivity is unclear. Two identified mutations in the β5 subunit, A142S and D150E, were not associated with alterations in susceptibility to proteasome inhibitors. Two mutations in the RPN10 subunit and one mutation in PF3D7_0808300 were common, but these were not associated with altered susceptibility to tested compounds. No polymorphisms were seen in the 19S PfRPT4 subunit, the α-type-6, subunit, or the fourth exon of the β6 subunit, in which mutations have previously been selected by proteasome inhibitors *in vitro* (10, 12, 22).

To further study the relevance of the β2 214F mutation, which was identified in Ugandan isolates, we assessed susceptibilities of culture adapted Ugandan strains to multiple proteasome

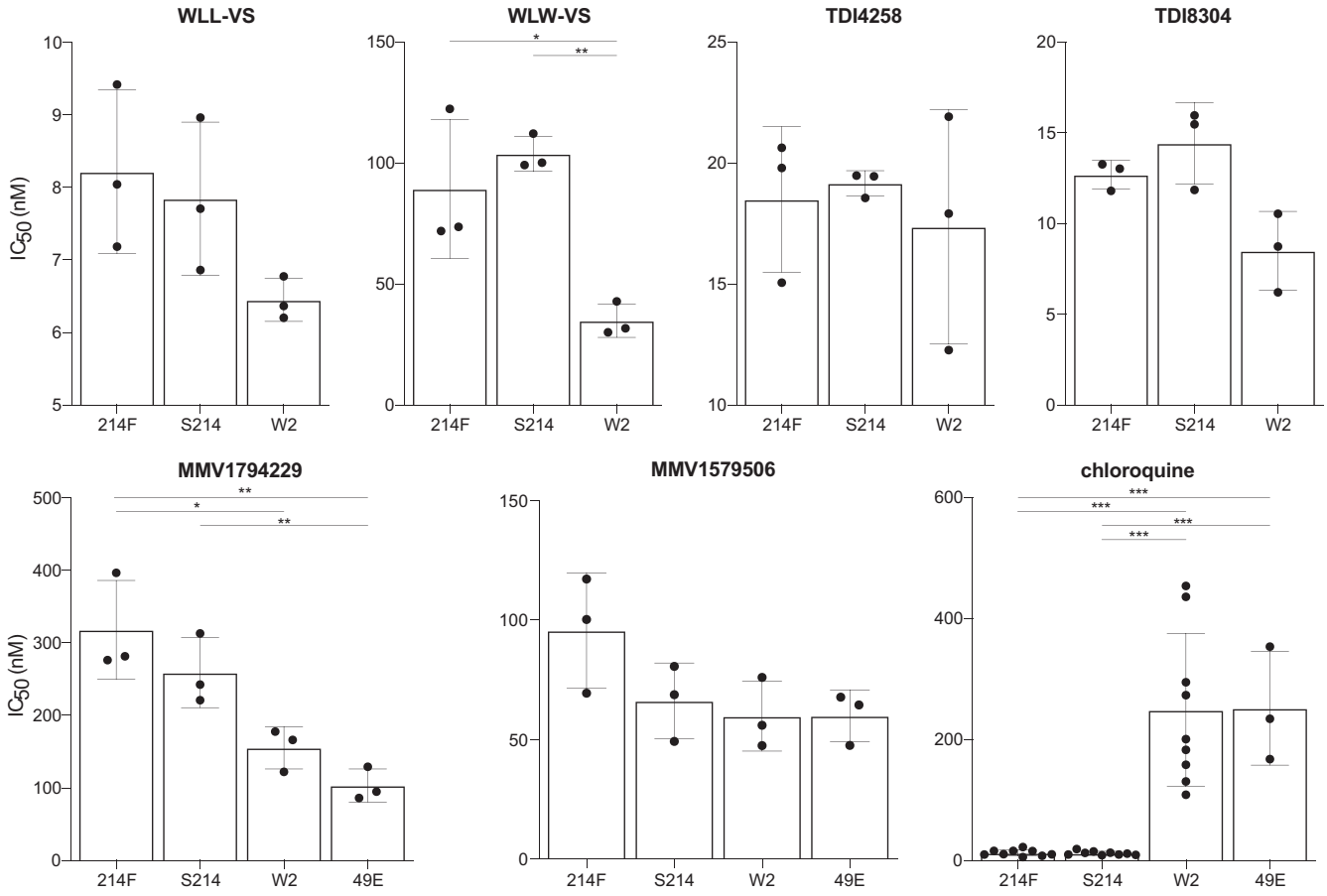


FIG 4 Susceptibilities of β2 mutant parasites to β2 and β5 subunit inhibitors. Each point represents an independent experiment, for a total of three independent biological replicates, with two technical replicates for β2 mutant (214F) and wild type (S214) Ugandan isolates, a laboratory mutant (49E) derived from V1/S K13^{C580Y}, and control strain W2. The histograms show mean IC_{50}s with standard deviations. Significant comparisons are indicated as * $<$0.5; ** $<$0.01; and *** $<$0.001.

inhibitors. β2 214F mutant parasites had decreased susceptibility to MMV1579506 and MMV1794229 compared to that of W2 strain and laboratory-selected β2 49E mutant parasites, but there were no significant differences in susceptibilities between β2 S214F wild type and mutant Ugandan strains. There were no significant differences in susceptibilities to other tested inhibitors between β2 wild type and mutant parasites.

This study had important limitations. First, different proteasome inhibitors were available for *ex vivo* analysis for only limited time frames, limiting our sample size for key compounds. One class, the peptide vinyl sulfones, was not available until after evaluation of field isolates, and therefore was only studied against selected culture adapted clones. Second, only small numbers of isolates with mutations in the β2 or β5 subunit were identified, limiting our ability to characterize the impacts of these polymorphisms. Third, most mutant isolates had mixed wild type/mutant genotypes, as typical in a region with high multiplicity of infection, further limiting our ability to characterize impacts of mutations.

In summary, multiple ethylenediamine and peptide boronate proteasome inhibitors demonstrated potent activity against Ugandan *P. falciparum* isolates. Modest variations in activities were generally not explained by polymorphisms seen in the β2, β5, RPN10, and PF3D7_0808300 subunits, although the β2 S214F mutation was associated with decreased *ex vivo* activity of two inhibitors in the few isolates with this mutation. Importantly, resistance-mediating mutations previously selected *in vitro* were not seen in Ugandan isolates. Overall, our results add confidence that, although studied proteasome inhibitors can select for resistance *in vitro*, they are likely to show consistently strong activity against *P. falciparum* isolates now circulating in East Africa.

MATERIALS AND METHODS

Sample collection. Between July 2017 and August 2020, subjects over 6 months of age presenting at the outpatient clinics of Tororo District Hospital, Tororo District or Masafu General Hospital, Busiu District diagnosed with uncomplicated falciparum malaria, confirmed by Giemsa-stained blood films, were enrolled after providing informed consent, as previously described (24). Blood was collected in heparinized tubes and spotted onto filter paper before treatment with artemether-lumefantrine, following national guidelines. The study was approved by the Makerere University Research and Ethics Committee, the Uganda National Council for Science and Technology, and the University of California, San Francisco Committee on Human Research.

Sources of proteasome inhibitors. TDI4258 (12, 21, 22), TDI8304 (12, 21, 22), TDI8239 (25), WZ32 (12, 21, 22) and MMV1794229 (19) were synthesized as previously reported. MMV1579506 and MMV1581599 were synthesized by Takeda Pharmaceuticals and provided by Medicines for Malaria Venture. WLW-VS and WLL-VS were synthesized as previously described (17) and kindly provided by Matthew Bogyo, Stanford University.

***Ex vivo* and *in vitro* drug susceptibilities.** Compounds were prepared as 10 mM stock solutions in dimethyl sulfoxide (except H_2O or 70% EtOH for chloroquine) and stored at −20°C. Working solutions were prepared within 24 h of susceptibility tests and stored at 4°C. Drug susceptibility assays utilized a 72 h SYBR green assay, as previously described (24). In brief, drugs were serially diluted 3-fold in complete media (RPMI 1640 medium supplemented with 25 mM HEPES, 0.2% NaHCO3, 0.1 mM hypoxanthine, 25 μg/mL gentamicin, and 0.5% AlbuMAX II [Invitrogen]). For *ex vivo* studies, the concentration ranges of the compounds were 10 μM to 0.51 nM for TDI-8304, TDI-8239, MMV1794229, and MMV1579506; 50 μM to 25 nM for MMV1581599; and 1 μM to 0.05 nM for TDI-4258 and WZ32. For the *in vitro* studies, the concentration ranges of the compounds were 5 μM to 0.25 nM for chloroquine, WLL, and WLW and 10 μM to 0.51 nM for TDI-4258, TDI-8304, MMV1794229. Parasite cultures (including the control laboratory strain W2, obtained from the Malaria Research and Reference Reagent Resource Center) and drug-free or parasite-free controls were added to the 96-well assay plates to reach a parasitemia of 0.2% and hematocrit of 2%, plates were incubated for 72 h at 37°C, and parasite growth was then assessed based on SYBR green fluorescence. *In vitro* drug susceptibility assays consisted of three biological replicates with two technical replicates per assay. Relative fluorescence intensity, measured at 485 nm excitation and 530 nm emission, was plotted against log drug concentration to determine the IC_{50} for each compound. Data were curve fit with a variable slope function using Prism 8.4 (GraphPad Software).

Culture adaptation of Ugandan isolates. Ugandan isolates were cultured for 2 to 3 weeks and, when robust growth was established, cryopreserved in 50% glycerolyte (Fenwal) solution and stored in liquid nitrogen. Subsequently, parasites were thawed using a stepwise process in 12% NaCl, 1.6% NaCl, and then 0.2% glucose/0.9% NaCl, and cultured in complete media at 2% hematocrit.

Characterization of *P. falciparum* genotypes. *P. falciparum* DNA was extracted from dried blood spots using Chelex-100 (26). The β2 subunit (PF3D7_1328100) was amplified by PCR with primers β2_F1 (ATGAAACTCGAATATATAAATATACTC) and β2_R1 (GGTATAACTTATGCATCTTCTACAG). The β5 subunit (PF3D7_1011400) was amplified by PCR with primers β5_F1 (ATGGTAATAGCAAGTGATG) and β5_R1 (AACATATTGATCCTTTTGTTCAGGA). Part of the fourth exon of the β6 subunit (PF3D7_0518300) was amplified using a nested PCR with primers β6_F1 (CAGAGAAGGAAAATGTAATAGAGCATG) and β6_R1 (GTCTCTTTCAGT TGCTGAAGTAATAG) for round 1 and primers β6_F2 (ACTGTCATTGGCTTAACTGGT) and β6_R2 (GGAACCACTAC CAACACAGGA) for round 2. Sequences of PCR products were determined using dideoxy sequencing (Eurofins Genomics), and sequences were analyzed with CodonCode Aligner (CodonCode Corporation) against the 3D7 reference genome. In addition, the β2 subunit sequences of 769 isolates, β5 subunit sequences of 785 isolates, 19S PfRPT4 subunit sequences of 893 isolates, alpha-type-6 subunit sequences of 893 isolates, RPN10 subunit sequences of 833 isolates, and PF3D7_0808300 sequences of 627 isolates were analyzed using MIP capture and deep sequencing, with library preparation and sequencing as previously reported (27). Subunit specific probes for this analysis were designed using MIPTools software (v.0.19.12.13; https://github.com/bailey-lab/MIPTools) (Table S1). The Miplicorn program (https://github.com/bailey-lab/miplicorn) was used to analyze raw sequencing data and call variants.

Construction of a model of the β2 subunit. A model of the β2 subunit was constructed with PyMOL using the published structure of the Pf20S proteasome (PDB model number: 6MUV; *P. falciparum* 20S proteasome which includes the β2 subunit and two PA28 activators). The mutagenesis tool was used to visualize the mutations observed in the Ugandan isolates.

Statistical analysis. The Spearman-rank test was used to assess correlations of results for individual isolates between compounds. The Wilcoxon test was used to assess associations between IC_{50}s and genotypes. One-way ANOVA was used to detect overall differences in *in vitro* susceptibilities of Ugandan isolates and laboratory strains. *Post hoc* analysis was conducted using pairwise t-tests with Bonferroni correction to determine which matched pairs were significantly different from one another. Statistical analysis was conducted using Prism 8.4 and R v.3.4.4. All tests were two-tailed and considered statistically significant at $P < 0.05$.

Data availability. Raw sequencing reads are available in the NCBI Sequence Read Archive under accession number (PRJNA850445 and ON042495-ON042749, ON165416-ON165513, ON661874-ON661996). MIP probes and PCR primers used in this study are listed in Table S1. MIPWrangler (https://github.com/bailey-lab/MIPWrangler) and MIPTools (https://github.com/bailey-lab/MIPTools) software is available on GitHub. All additional data are available on request from the authors (philip.rosenthal@ucsf.edu).

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.2 MB.

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