Bioorganic & Medicinal Chemistry Letters 20 (2010) 6688–6695

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

The antiplasmodial activity of norcantharidin analogs

Joanna Bajsa [a,*], Adam McCluskey [b], Christopher P. Gordon [b], Scott G. Stewart [b], Timothy A. Hill [b], Rajnish Sahu [c], Stephen O. Duke [a], Babu L. Tekwani [c]

[a] USDA, ARS, Natural Products Utilization Research Unit, University, MS 38677, USA
[b] Chemistry, School of Environmental and Life Sciences, The University of Newcastle, NSW 2308, Australia
[c] National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, MS 38677, USA

ARTICLE INFO

Article history:
Received 7 July 2010
Revised 31 August 2010
Accepted 1 September 2010
Available online 7 September 2010

Keywords:
Plasmodium falciparum
Antimalarial drugs
Cantharidin
Protein phosphatase inhibitors

ABSTRACT

The antiplasmodial activities of sixty norcantharidin analogs were tested in vitro against a chloroquine sensitive (D6, Sierra Leone) and chloroquine resistant (W2) strains of *Plasmodium falciparum*. Forty analogs returned IC_{50} values <500 μM against at least one of the *P. falciparum* strains examined. The ring open compound **24** ((1*S*,4*R*)-3-(allylcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid) is the most active aliphatic analog (D6 IC_{50} = 3.0 ± 0.0 and W2 IC_{50} = 3.0 ± 0.8 μM) with a 20-fold enhancement relative to norcantharidin. Surprisingly, seven norcantharimides also displayed good antiplasmodial activity with the most potent, **5** returning D6 = 8.9 ± 0.9 and W2 IC_{50} = 12.5 ± 2.2 μM, representing a five-fold enhancement over norcantharidin.

Malaria, the disease caused by the parasite *Plasmodium* spp., still contributes significantly to human mortality, especially in developing countries. Poverty and lack of proper medical care intensify the strong impact of this disease. Every year over one million people die because of an infection by this protozoan parasite.[1,2] This situation is complicated by *Plasmodium*'s complex life cycle with host and development stage changes and our limited knowledge of the molecular intricacies of these processes. Many researchers are currently examining antimalarial vaccines and drugs. However, there remains a lack of efficient vaccinations, and *Plasmodium* spp. easily evolve resistance to intensively used antimalarial drugs such as chloroquine and sulphadoxine-pyrimethamine.[3] Therefore, the discovery of new antimalarial leads with novel targets of action is an important goal.

In traditional Chinese medicine, dried beetle bodies have been used as a medicine for over 2000 years to cure hepatoma and esophageal carcinoma.[4,5] Cantharidin **1** is an active compound isolated from blister and oedemerid beetles.[6] It comprises a 7-oxabicyclo[2.2.1] frame fused with a 2,3- succinic anhydride moiety and two methyl groups. Cantharidin shows good anticancer activity; for example, recent reports indicated that this compound is an apoptosis inducer in multiple melanoma cells by interaction with proteins of the JAK/STAT pathway.[7] To date the high renal toxicity has precluded cantharidin's use in Western medicine.[5] This problem has promoted a search for less renal cytotoxic and more selective cantharidin analogs with a similar or improved anticancer profile. A number of analogs have been screened against various cancer cell lines such as breast, ovarian, lung, skin, prostate, leukemia, and colon.[8]

The most well-known analog of cantharidin is endothall, a dicarboxylic acid synthetic herbicide that had the best antiplasmodial activity among the commercial herbicides that we tested previously.[9] In addition to endothall, norcantharidin is the most well-known analog of cantharidin. This demethylated cantharidin derivative has lower nephrotoxicity than cantharidin,[10] and as such norcantharidin has been used as the lead compound in multiple investigations targeting the development of more potent and selective analogs.[8,11–14] Norcantharidin displays an about 10-fold activity reduction against cancer cell lines than cantharidin, but maintains cantharidin's favorable stimulation of white cell growth by bone marrow (in contrast to other anticancer drugs that readily induce myelosuppression). Most norcantharidin analogs can be classified as: (a) ring opened ester; (b) ring opened amides; or (c) ring closed imides, the norcantharimides.[8,10–14]

Cantharidin and its analogues have attracted significant interest, as they are the simplest members of the okadaic acid class of serine/threonine protein phosphatase (PPP) inhibitors. Cantharidin, norcantharidin and endothall are potent PPP inhibitors especially of mammalian and plant PP1 and PP2A (IC_{50} = 1.8 and 0.2 μM; 2.0 and 0.4 μM; 4 and 0.09 μM, respectively).[10–17] Except for the methyl groups, and limited synthetic variations associated with the anhydride moiety are permissible, all other structural elements have been shown to be crucial to the inhibitory activity of this compound. Removal of the 5,6-ethylbridge also renders

* Corresponding author. Tel.: +1 662 915 1010; fax: +1 662 915 7062.
E-mail addresses: jbajsa@olemiss.edu (J. Bajsa), btekwani@olemiss.edu (B.L. Tekwani).

0960-894X/$ - see front matter © 2010 Published by Elsevier Ltd.
doi:10.1016/j.bmcl.2010.09.004

cantharidin analogs inactive.[18] Bertini et al. noted that in a cantharidin-PP5 co-crystal, cantharidin sits in the catalytic site in its dicarboxylic acid form coordinating with one metal (either a Zn^{2+} or Mn^{2+}) via three oxygen atoms: one from each of the carboxylates, and the other from the 7-oxa moiety. This explains the crucial nature of the ethereal oxygen.[19] The two carboxylate moieties are in close proximity and expel all active site water molecules. Interestingly, in all instances a double conformation was observed (1:1 ratio) in which the bound structure only differed by the relative orientations of the carboxylate moieties. No double conformation was observed with norcantharidin, suggesting that the methyl groups, as suggested by Gauss et al., act as a conformational lock.[20]

Protein phosphatases and kinases complement one another by keeping equilibrium between dephosphorylated and phosphorylated forms of particular proteins. In this manner they regulate a substantial number of cellular processes such as gene expression, DNA replication, cellular proliferation and differentiation, apoptosis, etc.[21,22] Disturbance of PPP levels can lead to cell death in many cases.[23,24] Protein phosphatases have extremely sophisticated systems of their regulation of gene expression at both the transcription and translation levels. In spite of intense research, these regulation processes are still not well understood. Deregulation of protein phosphatase functioning contributes to a number of diseases such as tumor development, Alzheimer's disease and lupus erythematosus.[25] Examples of deregulating agents are viral proteins which act as modulators of both activity and specificity of protein phosphatases; that is, polyoma virus small T antigen and middle T antigen.[20]

The *Plasmodium falciparum* genome encodes six serine/threonine protein phosphatases that may be potential targets of cantharidin and/or norcantharidin, that is, PfPP1 (AAN36754), PfPP2A (CAB38970), PfPP2A-like (CAD51958), PfPPα (AAN37243), PfPPβ (CAD51935) and PfPP5 (CAD52675); their amino acid sequences contain a highly conservative PPP catalytic site $GDXHG(X)_nGDXVDRG(X)_nRGNHE$ (Fig. 1).[26] Despite very high amino acid sequence identity of the catalytic site between species, some sequence differences occur that could affect substrate preference, for example, PfPPα sequence contains a 15 amino acid long insert between the GDXHG(X) and GDXVDRG sequences (Fig. 1). In the future, this fact can be applied towards the design of compounds specific only to *Plasmodium* serine/threonine protein phosphatases.

The intricate *Plasmodium* life cycle has hampered research on functions and roles of *Plasmodium* PPP. Recent studies have shown that the function of PP1 is essential for *P. falciparum* during release of mature merozoites from erythrocytes. Inhibition of PfPP1 caused hyperphosphorylation of PfSBP1 (*P. falciparum* skeleton binding protein 1) and subsequently reinforcement and stabilization of the RBC membrane.[27] PfSBP1 is involved in transport of the major malaria virulence antigen PfEMP1 on the surface of RBCs.[28]

Recently, extensive studies have been conducted on the synthesis of several novel norcantharidin analogs with the main goal of obtaining compounds with higher activity and specificity.[10–14,18,29] In the present investigation we were keen to examine the ability of a select range of norcantharidin analogs (60 analogs as shown in Tables 1–3) inhibit the D6 and W2 strains of *P. falciaparum*. The *P. falciparum* D6 and W2 strains represent one of the most common and virulent forms of *Plasmodium* spp.[30]

The norcantharidin analogs examined can be separated into two broad classes: (1) the human PP1 and PP2A (HsPP1 and HsPP2A) inactive norcantharimides (Table 1). The norcantharimides possess a N-substituted imide moiety and are often known as the ring closed variants. (2) The human PP1 and PP2A active ring opened acid-amide norcantharidins (Tables 2 and 3). These norcantharidin analogs are further subdivided into two groups, firstly the aliphatic (Table 2) which possess alkyl amides terminating in a range of alkyl functional groups. The second grouping is the aromatic substituted norcantharimides where the primary functional group is an anilide (or an alkyl aromatic) (Table 3). We have previously reported inhibitory activity of these compounds against human catalytic subunits of PP1, PP2A and also for anticancer activity (human cancer cell lines: HT29, SW480 (colon), MCF-7 (breast), A2780 (ovarian), H460 (lung), A431 (skin), DU145 (prostate), BE2-C (neuronal), and SJ-G2 (glioblastoma)) of these compounds.[8,10,11,16,29] We compared the human phosphatases data described earlier to our antiplasmodial results introduced in this paper. Facing a lack of data about norcantharidin analogs inhibitory activity against plasmodium phosphatases, the results of human-host PPP activity bring a potentially useful perspective.

Our lead compound in this evaluation, norcantharidin displayed IC_{50} values of 60 ± 0.0 and 50 ± 0.0 μM against the *P. falciparum* strains D6 and W2, respectively.[30] This compares reasonably favorably with the more toxic, and hence less viable, cantharidin with activities of 9.0 ± 0.8 and 9.0 ± 0.0 μM against the D6 and W2 strains, respectively (Table 1). As can be seen from the data presented in Table 1, seven norcantharimides **5**–**10** and **18** return IC_{50} values of <500 μM (Table 1). Analogs **5** and **18** deserve particular note, showing IC_{50} values of D6 8.9 ± 0.9, W2 12.5 ± 2.2 μM; and D6 24.0 ± 4.8, W2 25.0 ± 0.0 μM, representing a five-fold enhancement in activity relative to norcantharidin. The other active analogues provide crucial structure–activity data with **3**–**10** showing a significant correlation between alkyl chain length, their *c*Log *P* value and growth inhibition of *P. falciparum* (Table 1). Elongation of **5**'s butyl chain to hexyl (**6**) and octyl (**7**) reduced the antiplasmodial efficacy, although this may in part be due to reduced solubility and uptake in the assay media. Constraining the *N*-alkyl moiety in a cyclohexyl ring is less detrimental to activity (IC_{50} **10**

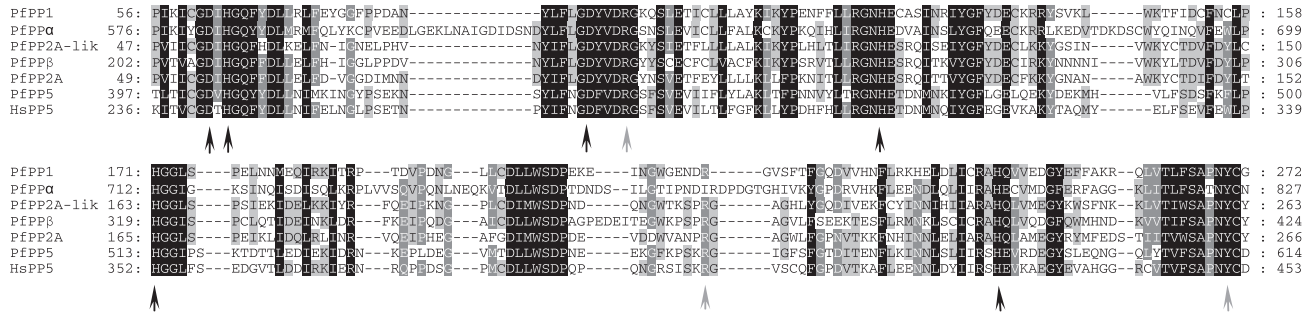


Figure 1. The alignment of *Plasmodium falciparum* serine/treonine protein phosphatases' and human PP5c[19] amino acid sequences. Fragments of catalytic sites of PPP with high similarity are marked. Black arrows pointing at amino acids responsible for coordination of metal ions located at active site Asp242, His 244, Asp 271, Asn 303, His 352 and His 427. Cantharidin and norcantharidin interact with two metal ions and Arg 275, Tyr 451 and Arg 400 (grey arrows).[19]

Table 1
The inhibition of the D6 and W2 *Plasmodium falciparum* malarial strains, and calculated Log *P* (*c*Log *P*) values, of cantharidin **1**, norcantharidin **2** and norcantharimides **3–19**

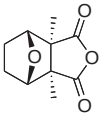
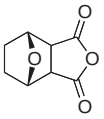
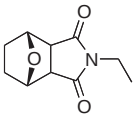
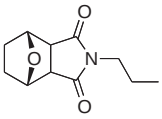
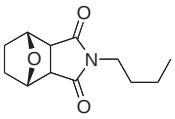
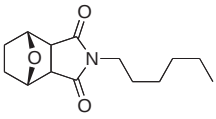
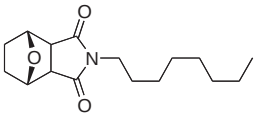
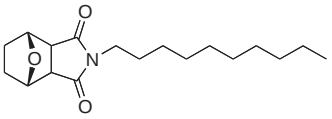
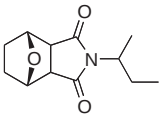
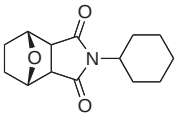
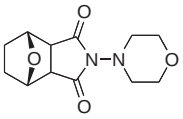
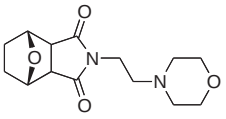
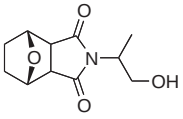
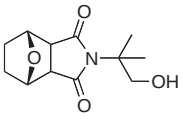
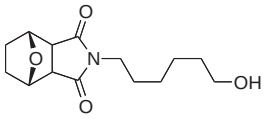
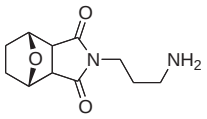
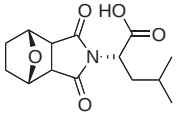
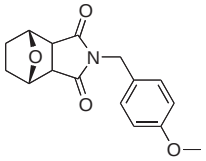
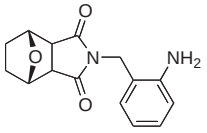
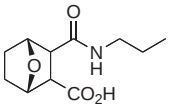
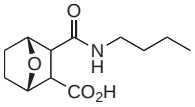
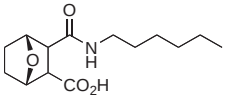
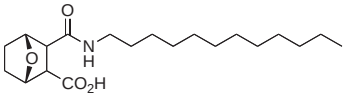
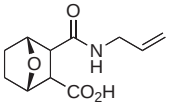
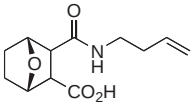
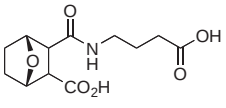
Compound	Structure	IC_{50}[a] (μM) Strain D6	IC_{50}[a] (μM) Strain W2	*c*Log *P*
Cantharidin **1**		9.0 ± 0.8	9.0 ± 0.0	−0.21
Norcantharidin **2**		60 ± 0.0	50 ± 0.0	−0.76
3		>500	>500	−0.21
4		>500	>500	0.26
5		8.9 ± 0.9	12.5 ± 2.2	0.72
6		175 ± 20.4	90 ± 48.9	1.65
7		225 ± 61.2	250 ± 40.8	2.58
8		375 ± 61.2	141 ± 48.8	3.50
9		225 ± 20.4	125 ± 20.4	0.66
10		140 ± 49.0	55 ± 20.4	0.94
11		>500	>500	−2.19
12		>500	>500	−1.04
13		>500	>500	−0.76
14		>500	>500	−0.61

Table 1 (*continued*)

Compound	Structure	IC_{50}[a] (μM) Strain D6	IC_{50}[a] (μM) Strain W2	*c*Log *P*
15		>500	>500	0.7
16		>500	>500	−1.23
17		>500	>500	0.11
18		24 ± 4.8	25 ± 0	0.62
19		>500	>500	0.00

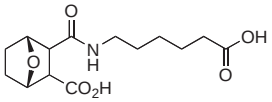
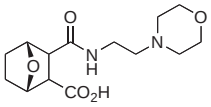
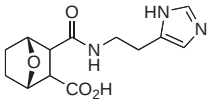
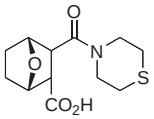
[a] Values are mean ± S.D. of three different experiments.

Table 2
The inhibition of the D6 and W2 *Plasmodium falciparum* malarial strains, human PP1 and PP2A and calculated Log *P* (*c*Log *P*) values, of ring opened aliphatic norcantharidin analogs **20**–**30**

Compound	Structure	HsPP1 IC_{50} (μM)	HsPP2A IC_{50} (μM)	IC_{50}[a] (μM) Strain D6	IC_{50}[a] (μM) Strain W2	*c*Log *P*
20		46 ± 4	18 ± 1	>500	>500	−0.23
21		70 ± 6	24 ± 5	>500	>500	0.23
22		35 ± 5	13 ± 0	>500	390 ± 122	1.16
23		—[b]	—	70 ± 24.4	106 ± 47	3.94
24		40 ± 2	10 ± 4	3 ± 0	3 ± 0.8	−0.53
25		—	—	>500	>500	−0.06
26		15 ± 2	3.3 ± 0.2	>500	>500	−1.12

(*continued on next page*)

Table 2 (*continued*)

Compound	Structure	HsPP1 IC_{50} (μM)	HsPP2A IC_{50} (μM)	IC_{50}[a] (μM) Strain D6	IC_{50}[a] (μM) Strain W2	*c*Log *P*
27		14 ± 1	3.5 ± 0.4	260 ±	>500	−0.19
28		30 ± 3	43 ± 1	>500	>500	−1.53
29		*(35 ± 6)*	*(56 ± 9)*[c]	307 ± 108	267 ± 126	−1.27
30		3.2 ± 0.0	5.1 ± 0.4	250 ± 45	204 ± 65	−0.50

[a] Values are mean ± S.D. of three different experiments.
[b] Insoluble in the protein phosphatase assay media at 40 mM stock concentration.
[c] Values in parentheses and italics are percentage inhibition values at 100 μM drug concentration.

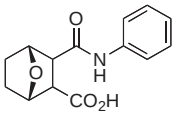
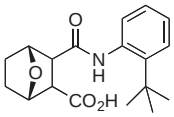
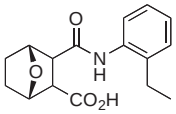
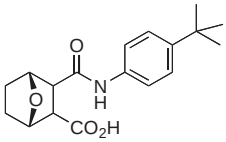
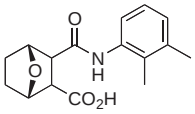
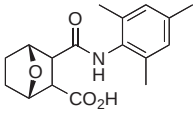
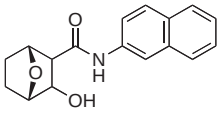
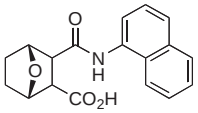
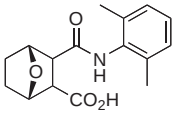
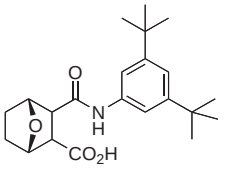
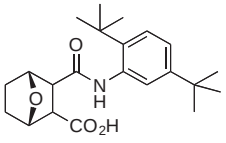
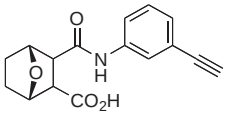
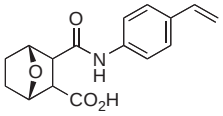
D6 140 ± 49; W2 55.0 ± 20.4 μM), but still effects a significant potency reduction compared with **5**. The observation of any antiplasmodial activity was surprising, as we have previously noted these norcantharimides to be bereft of inhibitory activity against human PP1 or PP2A.

Next we turned our attention to the PP1 and PP2A active ring opened norcantharidin analogs (Tables 2 and 3). Examination of the data in Table 2 highlights five analogues **3**–**5**, **8**, **10** and **11** with antiplasmodial IC_{50} values <500 μM. In this series, it is evident that that antiplasmodial activity is gained as a function of increasing alkyl chain length with *N*-propyl and *N*-butyl showing no activity (**20** and **21**), but *N*-hexyl displaying low levels of activity against the W2 strain (**22**, IC_{50} 390 ± 122 μM), and further elongation to *N*-dodecyl affords activity approaching that observed for the parent norcantharidin (**23**, IC_{50} D6 70 ± 24, W2 106 ± 47 μM). In this series, **24** is clearly the stand out compound with D6 and W2 IC_{50} values of 3 ± 0 and 3 ± 0.8 μM, respectively, a 20-fold enhancement relative to norcantharidin. The difference in activities between **20** and **24** is striking with the *N*-propyl to *N*-allyl modification affording a >150-fold potency increase. Equally striking is the difference between **24** and **25** with the *N*-allyl to *N*-butenyl moiety affording a >150-fold potency reduction. This is strongly suggestive that the resonance stabilizing capacity of the allyl moiety is responsible for this increased antiplasmodial activity. In this series, **29** appears to represent the intermediate species (between **24** and **25**) with an increased chain length terminating in an imidazole moiety which has a reduce capability of resonance stabilization. Terminal carboxylates (**26** and **27**) render these analogs inactive against D6 and W2 *P. falciparum* strains. We also note no obvious correlation with inhibition of human serine/threonine protein phosphatases and levels of antiplasmodial activity. This does not preclude that compound's ability to access the key proteins is often the limiting factor. However, this was not surprising, as the plasmodium pool of serine/threonine phosphatases may be comprised of enzymes with different susceptibility to analogs evaluated.

A considerable number (twenty seven) of the ring opened aromatic norcantharidins (Table 3) display modest levels of activity, but none are more potent than either **24** or the parent cantharidin and norcantharidin. We have noted similar trend in our past examination of the cytotoxic effect of related norcantharidin analogues.[8,10,11,16,18,29] Only compounds **40** and **41** retained minor activity in the D6 strain (59 ± 29 and 40 ± 8 μM). In both cases, the compounds posses two *tert*-butyl substituents and have the highest *c*Log *P* values of this series, excepting **50**, which interestingly shows retention of antiplasmodial activity (W2 IC_{50} = 70 ± 8.7 μM). Analogue **50** is five times more potent than its shorter chain counterpart **49** (octyl vs pentyl), which also has a correspondingly lower *c*Log *P* value. We note that the majority of the antiplasmodally active aromatic analogs are also modest to good protein phosphatase inhibitors (either HsPP1, or HsPP2A or both), and those displaying the highest antiplasmodial activity also posses a hydrophobic substituent.

Based on the X-ray structure of human PP5, Bertini et al. demonstrated that cantharidin and norcantharidin bind in the active site of PPPs.[19] In both instances these small molecules display an excellent fit within a small largely hydrophobic pocket defined by the side chains of Tyr272, Val429, and Phe260 and the backbone atoms of Gln242. The positively charged Arg89 and Arg214 side chains act as gatekeepers of this hydrophobic cavity with the additional role of stabilizing the two metal coordinated carboxylate groups. All our analogs in Tables 2 and 3 retain key features that play indispensable roles in properly aligning of cantharidin in the enzymes active site, that is, the 7-oxa bridging etheral oxygen and C3 carboxylate substituent. We note here, as previously, only one carboxylate is required to retain PPP inhibition. The additional side chain moieties facilitate differing levels of inhibitor engagement with key amino acids on the periphery of the catalytic site. This is also in keeping with the known modes of action of the larger toxins of the okadaic acid class of compounds. The C3 carboxylate group of our norcantharidin analogs is most likely acting in a similar manner to the carboxylate group of okadaic acid (OA) which can mimic the phosphate bridging the metal ions. Besides the phosphate-binding pocket, the enzyme binding site consists of a hydrophobic groove responsible for substrate recognition, as evidenced in the structure reported by Bertini et al.[19] Structures of many phosphatases inhibitors like OA, tautomicin, mycrocistin and calyculin contain highly hydrophobic ends with high affinities for the hydrophobic domains of enzymes. The allyl moiety of compound **24** reinforces the phosphate-binding in the pocket. Our results that show improvement of inhibitory activity by structural alterations of cantharidin suggest that even better antimalarial analogs can be produced.

Table 3
The inhibition of the D6 and W2 *Plasmodium falciparum* malarial strains, HsPP1, HsPP2A and calculated Log *P* (*c*Log *P*) values, of ring opened aromatic norcantharidin analogs **31**–**62**

Compound #	Structure	HsPP1 IC_{50} (μM)	HsPP2A IC_{50} (μM)	IC_{50}[a] (μM) Strain D6	IC_{50}[a] (μM) Strain W2	*c*Log *P*
31		24 ± 2.8	7.7 ± 0.8	300 ± 82	363 ± 97	0.45
32		*(16 ± 5)*[b]	*(9 ± 0.3)*	>500	>500	1.97
33		51 ± 5.2	24 ± 3.3	360 ± 57	400 ± 8	1.12
34		10 ± 0	11 ± 1	405 ± 45	>500	1.97
35		*(62 ± 7)*	*(84 ± 5)*	260 ± 33	220 ± 28	1.08
36		*(23 ± 4)*	*(63 ± 19)*	340 ± 131	>500	1.39
37		14 ± 2	15 ± 1	280 ± 16	257 ± 4.7	1.63
38		*(73 ± 1)*	*(79 ± 4)*	210 ± 73	193 ± 37	1.63
39		*(24 ± 9)*	*(68 ± 6)*	306 ± 136	290 ± 0	1.08
40		8.2 ± 2.5	8.2 ± 0.7	59.3 ± 28.7	176 ± 102	3.48
41		—[c]	—	40 ± 8.1	205 ± 89.5	3.48
42		20 ± 5.0	16 ± 1.0	193 ± 41.0	194 ± 31	0.76
43		23 ± 0.0	11 ± 1.0	>500	400 ± 81	1.05

(*continued on next page*)

Table 3 (*continued*)

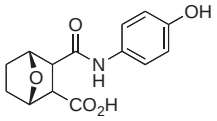
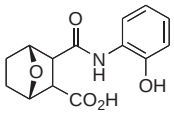
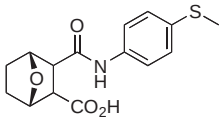
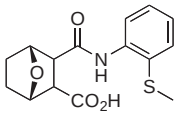
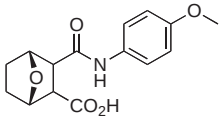
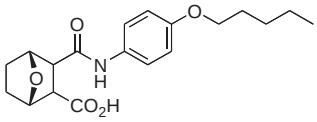
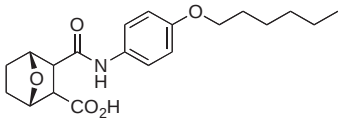
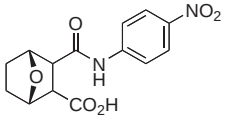
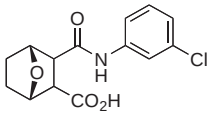
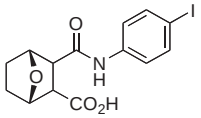
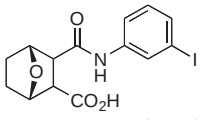
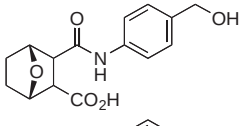
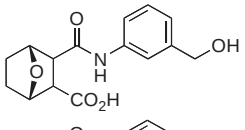
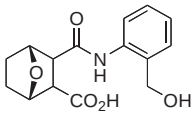
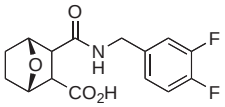
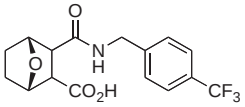
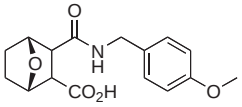
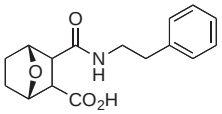
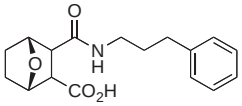
Compound #	Structure	HsPP1 IC_{50} (μM)	HsPP2A IC_{50} (μM)	IC_{50}[a] (μM) Strain D6	IC_{50}[a] (μM) Strain W2	*c*Log *P*
44		28 ± 3.2	7.7 ± 1.1	265 ± 12.2	333 ± 125	0.15
45		*(9.8 ± 11)*	*(33 ± 19)*	385 ± 94	380 ± 98	0.15
46		10 ± 4	14 ± 1.0	>500	>500	0.95
47		*(58 ± 4)*	*(76 ± 5)*	340 ± 131	>500	0.95
48		16 ± 0.5	16 ± 0.7	>500	>500	0.34
49		11 ± 4.9	15 ± 1.5	110 ± 8.1	333 ± 125	2.17
50		60 ± 15.3	20 ± 4.2	130 ± 57	70 ± 8.7	3.56
51		74 ± 13	23 ± 1.5	>500	393 ± 82	n.d.
52		31 ± 4.3	14 ± 0.9	250 ± 41	185 ± 18	1.06
53		33 ± 6.5	14 ± 1.2	230 ± 41	248 ± 84	1.38
54		44 ± 1.2	18 ± 0.7	217 ± 62	283 ± 111	1.38
55		15 ± 1.7	7.2 ± 2.0	160 ± 98	163 ± 31	−0.17
56		15 ± 0.3	7.0 ± 1.5	440 ± 49	390 ± 94	−0.17
57		*(30 ± 6)*	*(84 ± 3.2)*	275 ± 20	333 ± 118	−0.17

Table 3 (*continued*)

Compound #	Structure	HsPP1 IC_{50} (μM)	HsPP2A IC_{50} (μM)	IC_{50}[a] (μM) Strain D6	IC_{50}[a] (μM) Strain W2	*c*Log *P*
58		26 ± 2	26 ± 2	473 ± 38	>500	0.35
59		16 ± 3	20 ± 1	248 ± 85	433 ± 47	1.00
60		35 ± 5	13 ± 1	>500	>500	0.13
61		33 ± 0.4	29 ± 2	243 ± 33	300 ± 81	0.58
62		25 ± 0.0	25 ± 4	383 ± 85	230 ± 42	1.04

[a] Values are mean ± S.D. of three different experiments.
[b] Values in parentheses and italics are percentage inhibition values at 100 μM drug concentration.
[c] Insoluble in the protein phosphatase assay media at 40 mM stock concentration.

Acknowledgments

We thank Drs. Charles Cantrell and Cassia Mizuno for critical reviews of the manuscript.

References and notes

1. Van Dooren, G. G.; McFadden, G. I. *Nature* **2007**, *450*, 955.
2. Fidock, D. A.; Rosenthal, P. J.; Croft, S. L.; Brun, R.; Nwaka, S. *Nat. Rev. Drug Disc.* **2004**, *3*, 509.
3. Winzeler, E. A. *Nature* **2008**, *455*, 751.
4. Nicholls, L. C.; Teare, D. *Br. Med. J.* **1954**, *2*, 1384.
5. Wang, G. S. *J. Ethnopharmacol.* **1989**, *26*, 147.
6. Nikbakhtzadeh, M. R.; Dettner, K.; Boland, W.; Gäde, G.; Dötterl, S. *J. Insect Physiol.* **2007**, *53*, 890.
7. Sagawa, M.; Nakazato, T.; Uchida, H.; Ikeda, Y.; Kizaki, M. *Cancer Sci.* **2008**, *99*, 1820.
8. Hill, T. A.; Stewart, S. G.; Sauer, B.; Gilbert, J.; Ackland, S. P.; Sakoff, J. A.; McCluskey, A. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3392.
9. Bajsa, J.; Singh, K.; Nanayakkara, D.; Duke, S. O.; Rimando, A. M.; Evidente, A.; Tekwani, B. L. *Biol. Pharm. Bull.* **2007**, *30*, 1740.
10. McCluskey, A.; Ackland, S. P.; Bowyer, M. C.; Baldwin, L. M.; Garner, J.; Walkom, C.; Sakoff, J. A. *Bioorg. Chem.* **2003**, *31*, 68.
11. Hart, M. E.; Chamberlin, A. C.; Walkom, C.; Sakoff, J. A.; McCluskey, A. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1969.
12. McCluskey, A.; Sakoff, J. A. *Mini-Rev. Med. Chem.* **2001**, *1*, 43.
13. McCluskey, A.; Sim, A. T. R.; Sakoff, J. A. *J. Med. Chem.* **2002**, *45*, 1151.
14. Sakoff, J. A.; McCluskey, A. *Curr. Pharm. Des.* **2004**, *10*, 1139.
15. Ayaydin, F.; Vissi, E.; Mészáros, T.; Miskolczi, P.; Kovács, I.; Fehér, A.; Dombrádi, V.; Erdödi, F.; Gergely, P.; Dudits, D. *Plant J.* **2000**, *23*, 85.
16. Sakoff, J. A.; Ackland, S. P.; Baldwin, M. L.; Keane, M. A.; McCluskey, A. *Invest. New Drugs* **2002**, *20*, 1.
17. Thelin, W. R.; Kesimer, M.; Tarran, R.; Kreda, S. M.; Grubb, B. R.; Sheehan, J. K.; Stutts, M. J.; Milgram, S. L. *J. Biol. Chem.* **2005**, *280*, 41512.
18. Thaqi, A.; Scott, J. L.; Glibert, J.; Sakoff, J. A.; McCluskey, A. *Eur. J. Med. Chem.* **2010**, *45*, 1717.
19. Bertini, I.; Calderone, V.; Fragai, M.; Luchinat, C.; Talluri, E. *J. Med. Chem.* **2009**, *52*, 4838.
20. Gauss, C. M.; Sheppeck, J. E.; Nairn, A. C.; Chamberlin, A. R. *Bioorg. Med. Chem.* **1997**, *5*, 1751.
21. Janssens, V.; Goris, J. *Biochem. J.* **2001**, *353*, 417.
22. Van Hoof, C.; Goris, J. *Biochim. Biophys. Acta* **2003**, *1640*, 97.
23. Kinoshita, K.; Nemoto, T.; Nabeshima, K.; Kondoh, H.; Niwa, H.; Yanagida, M. *Genes Cells* **1996**, *1*, 29.
24. Gotz, J.; Probst, A.; Ehler, E.; Hemmings, B.; Kues, W. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 12370.
25. Katsiari, C. G.; Kyttaris, V. C.; Juang, Y. T.; Tsokos, G. C. *J. Clin. Invest.* **2005**, *115*, 3193.
26. Bajsa, J.; Duke, S. O.; Tekwani, B. L. *Curr. Drug Targets* **2008**, *9*, 997.
27. Blisnick, T.; Vincensini, L.; Fall, G.; Braun-Breton, C. *Cell. Microbiol.* **2006**, *8*, 591.
28. Cooke, B. M.; Buckingham, D. W.; Glenister, F. K.; Fernandez, K. M.; Bannister, L. H.; Marti, M.; Mohandas, N.; Coppel, R. L. *J. Cell Biol.* **2006**, *172*, 899.
29. Hill, T. A.; Stewart, S. G.; Ackland, S. P.; Gilbert, J.; Sauer, B.; Sakoff, J. A.; McCluskey, A. *Bioorg. Med. Chem.* **2007**, *15*, 6126.
30. *Assay procedure*: Test compounds were synthesized in-house according to published procedure (see Refs. 8,10,16,29). Cantharidin, norcantharidin, and chloroquine (CQ) were obtained from Sigma-Aldrich. The 96-well microplate antiplasmodial bioassay is based on evaluation of the effect of the compounds on growth of asynchronous cultures of *P. falciparum*, determined by the assay of parasite lactate dehydrogenase (pLDH) activity as described previously.[9]