Bioorganic & Medicinal Chemistry 19 (2011) 1541–1549

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

New imidazolidinedione derivatives as antimalarial agents

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ARTICLE INFO

Article history:
Received 14 October 2010
Revised 7 December 2010
Accepted 13 December 2010
Available online 16 December 2010

Keywords:
Antimalarials
Imidazolidinedione
Preparation
Metabolic stability

ABSTRACT

A series of new *N*-alky- and *N*-alkoxy-imidazolidinediones was prepared and assessed for prophylactic and radical curative activities in mouse and Rhesus monkey models. New compounds are generally metabolically stable, weakly active in vitro against *Plasmodium falciparum* clones (D6 and W2) and in mice infected with *Plasmodium berghei* sporozoites. Representative compounds **8e** and **9c** showed good causal prophylactic activity in Rhesus monkeys dosed 30 mg/kg/day for 3 consecutive days by IM, delayed patency for 19–21 days and 54–86 days, respectively, as compared to the untreated control. By oral, **9c** showed only marginal activity in causal prophylactic and radical curative tests at 50 mg/kg/day × 3 and 30 mg/kg/day × 7 plus chloroquine 10 mg/kg for 7 days, respectively.

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1. Introduction

8-Aminoquinoline (8-AQ) antimalarials remain the most potent class of malaria drugs with radical curative activity. The therapeutic value of the 8-AQ as first line antimalarial drug, nevertheless, is compromised by the lethal hemolysis side effect in glucose-6-phosphate dehydrogenase (G6PD) deficient patients. Search of non-8-AQ new antimalarial drugs with prophylactic and/or radical curative activity to replace 8-AQ antimalarial drugs, such as primaquine (PQ) and tafenoquine (TQ), is the paramount interest of this laboratory in recent years.[1–4] Imidazolidinedione derivatives, such as compound WR182393 (IZ), is one of the very few classes of compounds with liver stage antimalarial activity. It was first prepared and tested in exoerythrocytic (EE) mouse model in early 1970s and was later found to have radical curative and causal prophylactic activity in Rhesus infected with sporozoites of *Plasmodium cynomolgi*.[5] The test material was later shown to be a mixture of the biguanide starting material, chloroproguanil, and two imidazolidinedione derivatives (**1** and **2**). The results of our efforts to identify each of the components in the mixture are detailed in a recent publication.[1] Subsequently, unambiguous new methods for synthesis of the pure imidazolidinediones **1**, and **2** were developed which facilitated the analogs synthesis and structure activity relationship (SAR) studies. The efforts lead to the discovery of carbamates **3** and **4** which showed superior prophylactic activity to the lead compounds in Rhesus monkeys.[2–4] The structures of the two key active components (**1** and **2**) and their carbamate derivatives (**3** and **4**) are shown in Figure 1.

Carbamate **3**, the most active compound of this class, protects monkeys at a dose of 10 mg/kg/day for 3 days by im. Nevertheless, the IZ derivatives showed very weak or no activity against cell growth of *Plasmodium falciparum* clones (W-2 and D-6) and *Plasmodium berghei* in mice. To the best of our knowledge, this is the first class of antimalarial agents possessing activity against liver stage malaria exclusively. Given by oral, however, compound **3** only delayed patency (first day the parasite can be detected in blood smears after infection) of the treated monkeys for 3 days as compared to the untreated animals. The lacking of oral activity of carbamates **3** and **4** in Rhesus tests was first thought due to their poor bioavailability as a result of hydrolysis of carbamate group in stomach acid leading to generation of insoluble parent compounds **1** or **2**. Chemically stable IZ-carboxamide derivatives (**5** and **6**) were then prepared. However, the electron inductive effects of the carboxamide groups made the imidazolidinedione ring of **5** and **6** liable to hydrolysis and consequently leading to formation of *s*-triazines **7**.[4] The *s*-triazine formation was first thought to be a bioactivation process of the IZ-carboxamide derivatives, but was later found to be a chemical inactivation route. As a result, the carboxamide derivatives (**5** and **6**) are in general not as active as the carbamate analogs **3** and **4**.[4]

In this study, a series of chemically stable *N*-alkoxyl- (**8**) and *N*-alkyl- (**9**) derivatives of compound **1** (Fig. 2) was synthesized. Alkylamino- and alkoxylamine- groups are chemically stable though may be subjected to metabolic dealkylation. Alkoxylamino group has been used as a masking group in the design of amidine pro-drugs.[6] The new compounds prepared in this study are expected to be more stable chemically and/or metabolically, resulting in better antimalarials than carbamates (**3** and **4**) and carboxamides (**5** and **6**).

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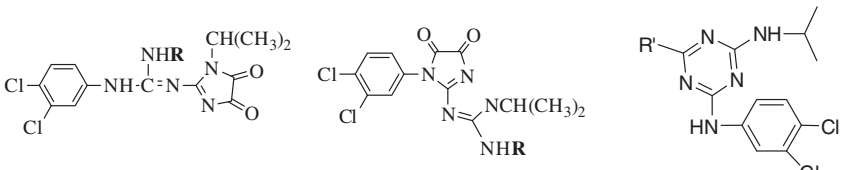
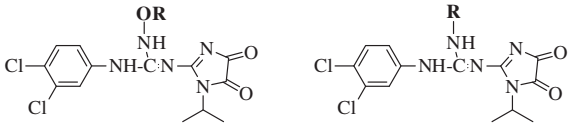
0968-0896/$ - see front matter Published by Elsevier Ltd.
doi:10.1016/j.bmc.2010.12.028



1	**R** = H	**2**	**R** = H
3	**R** = -COOC(CH$_3$)$_3$	**4**	**R** = -COOCH$_2$CH(CH$_3$)$_2$
5	**R** = -CONHR'	**6**	**R** = -CONHR'

7

Figure 1. Active components of WR182393 (**1** and **2**) and derivatives (**3**–**6**).



8 **9**

R = alkyl, aryl, alkylaryl, aminoalkyl et al

Figure 2. *N*-Alkyl and *N*-alkoxyl derivatives of compound **1**.

2. Chemistry

The preparation of the new compounds for this study is straightforward. Compounds **8a**–**e** and **9a**–**p** were prepared by heating of 1-isopropyl-2-methylsulfanyl-1*H*-imidazole-4,5-dione (**10**) with *N*-alkoxyl-3,4-dichlorophenylguanidine (**12a–e)** or *N*-alkyl-3,4-dichlorophenylguanidine (**13a**–**n**) in chloroform in a sealed tube at 100 °C for 18–72 h as shown in Scheme 1. The *N*-alkyl- and *N*-alkoxyguanidines were prepared in turn by either treating 1-(3,4-dichlorophenyl)-2-methylisothiourea hydroiodide (**11**) (method 1), or (3,4-dichlorophenyl)-cyanamide (**14**) (method 2) with alky- or alkoxyl-amines.[2,7–13] Synthesis of the starting materials, compound **1** and isopropyl-2-methylsulfanyl-1*H*-imidazole-4, 5-dione (**10**), were described in our previous publications of this series.[1–4] The ^{1}H NMR spectra of the final products **8** and **9** in $CDCl_3$ indicated the formation of tautomers **8′** and **9′**, respectively. Two sets of proton NMR spectra were observed in analogs of **8** and **9**, which coalesced into a single spectrum upon addition of a drop of CF_3COOD. The ratio of the two tautomers is concentration and temperature dependent and is roughly 4:1 at room temperature.

Attempts were also made to prepare the *N*-alkyl analogs **9** by direct alkylation of compound **1** using relatively reactive benzyl bromide. As compound **1** is a biguanide derivative, direct benzylation of which could lead to a mixture of products with the benzyl group randomly located at N1 through N4 as shown in Scheme 2. Surprisingly, under the reaction conditions employed, only one benzylation product was isolated. Based on NMR data alone, it is difficult to pinpoint the location of the benzyl group in the new product without using X-ray crystallography technique. The single crystal X-ray diffraction data indicated that the benzylation occurred at N1 instead of the desired N2 position, giving exclusively *N*-benzyl-*N*-(3,4-dichloro-phenyl)-*N′*-(1-isopropyl-4,5-dioxo-4,5-dihydro-1*H*-imidazol-2-yl)-guanidine (**IZB**) as shown in Supplementary data [S1, Fig. 3].

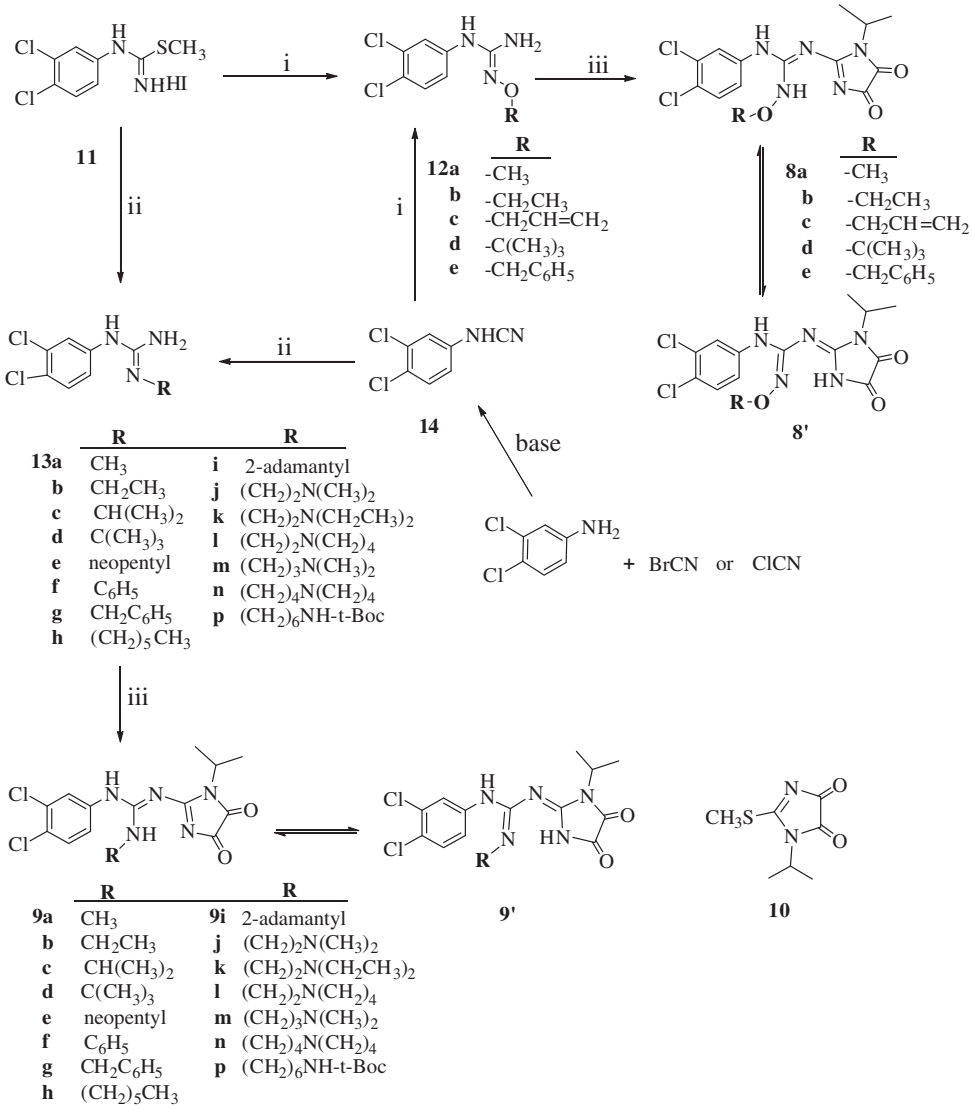
Likewise, although 3 possible isomers of alkylation products can be formed by heating *N*-alkoxyl or *N*-alkyl-3,4-dichlorophenylguanidine (**12** or **13**) and 1-isopropyl-2-methylsulfanyl-1*H*-imidazole-4,5-dione (**10**) under the conditions stated above, only one product was isolated. Contrary to the direct alkylation method described above which formed undesirable isomer **IZB**, reaction of **10** and **13a**–**p** provided the desired products in good yields. To unequivocally confirm the structure as the desired product, a single crystal X-ray crystallography on one of the representative compound **9m** was conducted and the result is shown in Supplementary data [S3, Fig. 4].

3. Results and discussion

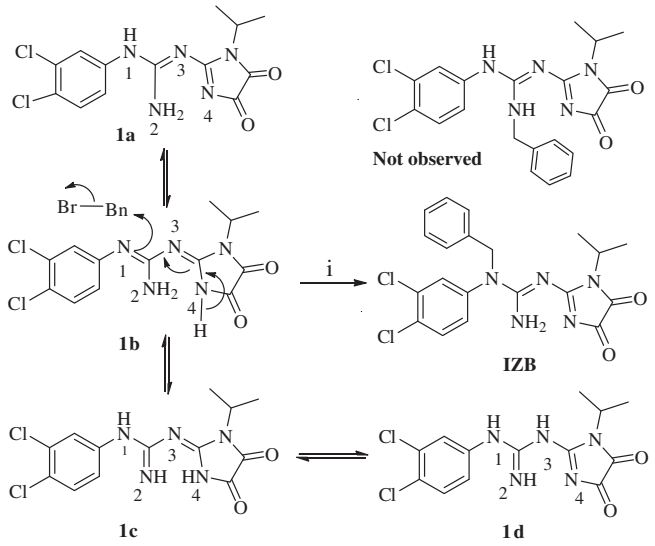
Antimalarial activities of the new compounds were assessed in clones of *P. falciparum* (W2 and D6), and in mice challenged with sporozoites of *P. berghei*. The results are shown in Table 1. Like the imidazolidinedione analogs reported earlier, the new compounds showed only weak in vitro activity against clones of *P. falciparum*, with IC_{50} in the range of 1 to >6 μg/ml. As was expected, most of the compounds are metabolically stable in mouse and human microsomal preparations with $t_{1/2}$ >60 min, except analogs **8e**, **9h**, **9i** and **9p** which showed a half-life ($t_{1/2}$) of 10 min or less in mice microsomal preparations. Chemically, these four compounds showed no substituents which are particularly vulnerable to enzymatic or chemical hydrolysis. Nevertheless, the *c*-Log *P* values of these four analogs are all greater than 5, a log value higher than the other compounds, indicating poorer water solubility. Most likely, compounds **8e**, **9h**, **9i** and **9p** may be precipitated out from the assay medium during the dilution process and thus giving a false high rate of hydrolysis in metabolic stability assay. In addition to the poor solubility, since **9P** is a *t*-Boc derivative, chemical hydrolysis of the *t*-Boc group during the experiment may also contribute to the observed instability results.

Like other IZ derivatives, the new compounds also showed weak or no activity in mice infected with sporozoites of *P. berghei*, dosed up to 320 mg/kg/day × 3 PO, except compounds **8a**–**c**, **9c** and **9e** which showed cures at 160 mg/kg/day × 3. Severe restless side effects and early death were observed among the mice received high dose of *N*-alkoxyl analogs, **8a**–**c**. Among the *N*-alkyl analogs tested, only **9c** and **9e** showed cures administered 160 mg/kg/day for three consecutive days. Unlike *N*-alkoxy derivatives, no sign of toxicity was observed among the mice treated with *N*-alkyl analogs **9a**–**p** up to 320 mg/kg.

Good in vitro, rodent and/or non-human primate test models are essential for lead optimization projects such as this study. Our efforts have been hampered by lacking of a good predictive exoerythrocytic (EE) mouse model and liver cell culture to conduct proper SAR studies to prioritize compounds for further testing in Rhesus. Two mouse models infected with, *P. yoelli* or *P. berghei* sporozoites, respectively, have been used to assess the liver stage antimalarial activities of the imidazolidinedione (IZ) derivatives.[1–4] Neither of these two models gave good predictive results in Rhesus. The former gave too many false positive and the latter gave too many false negative results. Lacking of good EE mouse model, the selection of new candidates for further test in Rhesus was based mainly on the previous observations that compounds of this class generally showed moderate to good IM activity, even though they showed poor activity in mice model. Therefore, only two new compounds, **8e** and **9c**, which represent alkoxy and alkyl



Scheme 1. Synthesis of *N*-alkyl and *N*-alkoxyl derivatives of compound **1**. Reagents and conditions: (i) $NH_2O\mathbf{R}$, EtOH, reflux, 18 h; (ii) $\mathbf{R}NH_2$, MeOH, 80–100 °C/sealed tube, 18 h; (iii) **10**, $CHCl_3$, 100 °C/sealed tube, 18–72 h.



Scheme 2. Benzylation of compound **1**. Reagents: (i) BnBr, TBAI, DMF.

derivatives, respectively, were assessed for causal prophylactic and radical curative activities in Rhesus. Prior to the monkey test, both compounds were confirmed no acute toxicity at 300 mg/kg/day for three consecutive days by sc administration, and lost no more than 5% body weight within 30 days after receiving the test compound.

Benzyloxy (**8e**) and isopropyl (**9c**) derivatives showed promising causal prophylactic activity in Rhesus test by IM. The former delayed patency of the treated monkeys for 19 days in one monkey and 21 days in the other at a dose of 30 mg/kg/day × 3 by IM (Table 2). In the same test, compound **9c** exhibited superior efficacy to **8e**, delayed patency for 54 days in one monkey and 86 days for the others. By oral administration, compound **9c** delayed the patency of the treated monkeys for only 3 days at 50 mg/kg/day × 3. Due to limited availability of naïve monkeys and modest IM activity, no attempt was made to evaluate **8e** for oral activity in Rhesus. In the same experiment, three clinical antimalarial drugs, primaquine, tafenoquine and malarone, were used as positive control at a human equivalent PO dose. To our surprise, none of these three first line antimalarial drugs protected the monkeys and showed only comparable activity to **9c**, delayed the patency of the treated monkeys for only 2–5 days (Table 2). The results

Table 1
Prophylactic activity, metabolic stability and in vitro efficacy

Compd #	Met. stability $t_{1/2}$ (min)		EE *P. berghei* (mg/k/day) × 3		*P. falciparum* IC_{50} (μg/ml)			*c*-Log *P*
	Mouse	Human	MAD[a]	MTD[b]	D6	TM[c]	W2	
8a	>60	>60	≤40	<40	>5.0	>5.0	>5.0	3.37
8b	>60	>60	160	<40	>5.0	>5.0	>5.0	3.90
8c	>60	>60	160	120	8.5	>15	6.7	4.20
8d	>60	>60	>160	≥160	2.75	3.36	1.14	4.60
8e	12.87	>60	>160	≥160	>5.0	>5.0	>5.0	5.14
9a	>60	>60	>320	≥320	>5.0	>5.0	>5.0	—
9b	>60	>60	>160	≥160	>5.0	>5.0	1.67	3.45
9c	>60	>60	160	≥160	>6.0	-	>6.0	3.80
9d	>60	>60	>320	≥320	0.72	1.36	1.15	4.14
9e	>60	>60	160	≥320	1.1	0.95	0.37	4.68
9f	>60	>60	>160	≥160	2.16	2.60	0.88	4.67
9g	>60	>60	>320	≥320	2.36	>5.0	1.30	—
9h	7.84	34.74	>160	≥160	1.35	2.80	2.62	5.57
9i	7.42	5.58	>320	≥320	0.81	2.07	2.68	5.81
9j	>60	>60	>160	≥160	>5.0	>5.0	>5.0	3.07
9k	17.65	31.51	>160	≥160	>5.0	>5.0	>5.0	4.13
9l	53.17	>60	>160	≥160	>5.0	>5.0	1.32	3.77
9m	>60	>60	>160	≥160	>5.0	>5.0	>5.0	3.29
9n	>60	>60	>160	≥160	1.55	>5.0	2.30	4.29
9p	10.93	12.47	>160	≥160	>5.0	>5.0	1.46	5.41

[a] MAD = minimum active dose.
[b] MTD = maximum tolerated dose.
[c] TM = TM91C235 (a clone of *P. falciparum*).

Table 2
Causal prophylactic activity of **8e**, and **9c** in *P. cynomolgi* sporozoites challenged Rhesus monkeys

Compd #	Dose[a] (mg/kg/day × 3)	Route	Delayed Patency (Day)	Patency[b] (Days Post-inoculation) (Day)
Control	N/A	IM[c]	Valid control	8
		PO[d]		8
8e	30	IM	19	27
			21	29
9c	30	IM	54	62
			86	94
	50	PO	3	11
			3	11
PQ[e]	1.78	PO	5	13
			5	13
TQ[f]	0.316	PO	3	11
			2	10
MA[g]	14	PO	2	10
			3	11

[a] Two monkeys per dose group.
[b] Patency = first day parasites can be detected in blood smears after infection.
[c] IM = intramuscular injection.
[d] PO = oral administration.
[e] PQ = primaquine.
[f] TQ = tafenoquine.
[g] MA = malarone.

indicated that **9c** cannot be ruled out as a potential prophylaxis for malaria infection and that the causal prophylactic protocol in Rhesus is in need of revalidation.

In a combined causal prophylactic/radical curative Rhesus model, compound **9c** exhibited no significant radical curative activity by oral administration, delayed the relapse time of treated monkeys for only 2–3 days versus untreated control at a dose of 30 mg/kg/day × 7 PO plus CQ 10 mg/kg/day × 7 PO (Table 3). In the same test, PQ at 1.78 mg/kg/day × 7 PO plus CQ at 10 mg/kg/day × 7 PO radically cured the infected monkeys.

As the life cycle of liver stage malaria in Rhesus is about 8 days and the plasma $t_{1/2}$ of the IZ compounds by oral administrations are generally less than 5 h. Thus, very low or no drug will remain in the plasma a day after dosing. Therefore, a 3–7 day treatment regimen is obviously insufficient to eradicate the hypnozoites unless the drug is very potent or long acting. Longer treatment (7–14 days) and higher dosing regimen (twice a day at 30–50 mg/kg/day) may be necessary to determine the oral activity of this class of compounds in Rhesus model.

4. Experimental

Melting points were determined on a Mettler FP62 melting point apparatus and are uncorrected. Analytical thin-layer chromatography (TLC) was performed using HPTLC-HLF normal phase 150 μ silica gel plates (Analtech, Newark, DE). Visualization of the developed chromatogram was performed by UV absorbance or by staining with iodine vapor. Liquid chromatography was performed using a Horizon HPFC System (Biotage, Charlottesville, VA) with Flash 25 M or 40 M cartridges (KP-Silica, 32–63 μm, 60 Å). Preparative TLC was performed using silica gel GF tapered uniplates (Analtech, Newark, DE). ^{1}H NMR and ^{13}C NMR spectra

Table 3
Radical curative activity of compound **9c** in relapsed Rhesus monkeys

Drug 1	Drug 2	Dose (mg/kg)	# Days treated	Route	Results	Relapsed[c] (Day)
Chloroquine (CQ) 10 mg/kg × 7, PO[a]	None	None	1 daily for 7 days	PO[a]	Relapsed	11 12
	9c	**30**	1 daily for 7 days	PO	Relapsed	15 14
	PQ[b]	**1.78**	1 daily for 7 days	PO	Radical cure	>100

[a] PO = oral administration.
[b] PQ = primaquine.
[c] Relapsed = first day parasitemia detected post treatment.

were recorded in deuterochloroform, unless otherwise noted, on a Bruker Avance 300 spectrometer (Bruker Instruments, Inc., Wilmington, DE). Chemical shifts are reported in parts per million on the δ scale from an internal standard of tetramethylsilane. When the compounds formed two tautomers, the chemical shifts with * indicated the peaks were shared by both tautomers in NMR spectra. Combustion analyses were performed by Atlantic Microlab, Inc. (Norcross, GA). Where analyses are indicated by symbols of the elements, the analytical results obtained were within ±0.4% of the theoretical values. Mass analysis based on electron impact (EI) was done on a Hewlett Packard (Agilent; Santa Clara, CA) 5973 Mass Selector adapted with a HPP7 Direct Insertion Probe (Scientific Instrument Services; Ringoes, NJ) using a steep temperature gradient at either low voltage (5 eV) to identify masses present or high voltage (70 eV) to obtain fragmentation data. An LC/UV–vis/Trap MS was also employed for purity analysis and chromophore properties. The system consisted of an Agilent 1100 Series LC–UV/vis system online with a ThermoFinnigan (now ThermoFisher; Waltham, MA) LCQ MS equipped with electrospray ionization (ESI) source. Samples were analyzed using shallow acetonitrile buffer gradients at low flow rate. Purity of the final products are >95%. Single crystal X-ray diffraction data was collected by Dr. Maren Pink, Director of the Molecular Structure Center, Indiana University, Bloomington, IN.

4.1. General procedure for the synthesis of *N*-alkoxy-*N*′-(3,4-dichlorophenyl)-guanidines 12a–e

A mixture of 3,4-dichlorophenyl-cyanamide (1.0 equiv) (**14**) and alkoxylamine HCl salt (2.0 equiv) in absolute ethanol was heated under reflux for 18 h. The solvent was removed under reduced pressure. Saturated aqueous Na_2CO_3 was added to the residue. The mixture was extracted with ethyl acetate three times. The organic extracts were combined, washed with brine, dried over Na_2SO_4, filtered, and the solvent was evaporated to dryness under reduced pressure. The residue was suspended in small amount of cold (~4 °C) CH_2Cl_2, filtered and washed with fresh CH_2Cl_2 to give white solid. Compounds **12a**–**e** was used for reactions without further purification.

4.1.1. *N*-Methoxy-*N*′-(3,4-dichlorophenyl)-guanidine (12a)

Following the general procedure, the title compound was obtained from methoxyamine HCl. Yield: 46%, mp 134 °C. ^{1}H NMR (CD_3OD): δ 3.69 (s, 3H), 7.11 (dd, J = 2.6 Hz and 8.8 Hz, 1H), 7.28 (d, J = 8.8 Hz, 1H), 7.56 (d, J = 2.6 Hz, 1H). MS (EI): m/z 233 $[M]^+$.

4.1.2. *N*-Ethoxy-*N*′-(3,4-dichlorophenyl)-guanidine (12b)

Following the general procedure, the title compound was obtained from *O*-ethylhydroxylamine HCl. Yield: 54%, mp 124 °C. ^{1}H NMR ($CDCl_3$): δ 1.28 (t, J = 7.0 Hz, 3H), 3.96 (q, J = 6.8 Hz, 2H), 4.00 (br s, 2H), 6.97 (br d, J = 7.4 Hz, 1H), 7.30 (s, 1H), 7.34 (d, J = 2.3 Hz, 1H). MS (EI): m/z 247 $[M]^+$.

4.1.3. *N*-Allyloxy-*N*′-(3,4-dichlorophenyl)-guanidine (12c)

Following the general procedure, the title compound was obtained from *O*-allylhydroxylamine HCl. Yield: 72%, mp 90 °C. ^{1}H NMR ($CDCl_3$): δ 4.39–4.45 (m, 2H), 4.55 (br s, 2H), 5.78 (br s, 1H), 5.24 (dd, J = 1.4 Hz, and 10.4 Hz, 1H), 5.33 (dd, J = 1.5 Hz, and17.2 Hz, 1H), 5.98–6.09 (m, 1H), 6.95 (dd, J = 2.7 Hz, and 8.7 Hz, 1H), 7.27 (d, J = 8.7 Hz, 1H), 7.33 (d, J = 2.7 Hz, 1H). MS (EI): m/z 259 $[M]^+$.

4.1.4. *N*-(*t*-Butyloxy)-*N*′-(3,4-dichlorophenyl)-guanidine (12d)

Following the general procedure, the title compound was obtained from *O*-*tert*-butylhydroxylamine HCl. Yield: 41%, mp 126 °C. ^{1}H NMR ($CDCl_3$): δ 1.30 (s, 9H), 6.96 (dd, J = 2.6 Hz, and 8.8 Hz, 1H), 7.27 (d, J = 8.8 Hz, 1H), 7.41 (d, J = 2.6 Hz, 1H). MS (EI): m/z 275 $[M]^+$.

4.1.5. *N*-Benzyloxy-*N*′-(3,4-dichlorophenyl)-guanidine (12e)

Following the general procedure, the title compound was obtained from *O*-benzylhydroxylamine HCl. Yield: 55%, mp 105 °C. ^{1}H-NMR ($CDCl_3$): δ 4.93 (s, 2H), 6.89 (dd, J = 2.3 Hz, and 8.6 Hz, 1H), 7.23–7.42 (m, 7H). MS (EI): m/z 309 $[M]^+$.

4.2. General procedure for the synthesis of *N*-alkyl-*N*′-(3,4-dichlorophenyl)-guanidines (13a–p)

Method 1: A mixture of 1-(3,4-dichlorophenyl)-2-methylisothiourea HI salt (**11**, 1.0 equiv) and alkylamine (5–10 equiv) in methanol was heated in a sealed tube at 80–100 °C for 18 h. The solvent and the excess amine were removed under reduced pressure. Saturated aqueous Na_2CO_3 solution was added to the residue and the mixture was extracted with EtOAc. The organic extracts were combined, washed with brine, dried over Na_2SO_4, filtered, and the solvent was removed under reduced pressure. The residue was suspended in cold (~4 °C) CH_2Cl_2, filtered and washed with fresh CH_2Cl_2 to give a white solid. The product was used for next step reactions without further purification.

Method 2: A mixture of 3,4-dichlorophenylcyanamide (1.0 equiv) (**14**) and alkyl or alkoxylamine (2.0 equiv) in absolute methanol was heated in a sealed tube at 100 °C for 18 h. The solvent and the excess amine were removed under the reduced pressure. The residue was purified by recrystallization in hexane/CH_2Cl_2 to give light brown solid.

4.2.1. *N*-Methyl-*N*′-(3,4-dichlorophenyl)-guanidine (13a)

Following the general procedure, method 1, the title compound was obtained by treatment of **11** with methylamine in absolute EtOH. Yield: 77%, mp 156 °C. ^{1}H NMR ($CDCl_3$): δ 2.87 (s, 3H), 3.72 (br s, 3H), 6.78 (d, J = 8.5 Hz, 1H), 7.05 (s, 1H), 7.32 (d, J = 8.5 Hz, 1H). Major tautomer: ^{13}C NMR (DMSO-d_6) δ 20.0, 29.5, 43.9, 125.7, 127.8, 130.7, 131.8, 136.3, 137.9, 159.0, 161.7, 168.2, 170.3; minor tautomer: ^{13}C NMR (DMSO-d_6) δ 20.3, 29.3, 127.2, 128.1, 131.0, 132.2, 160.3, 162.1, and 170.1. MS (EI): m/z 217 $[M]^+$.

4.2.2. *N*-Ethyl-*N*′-(3,4-dichlorophenyl)-guanidine (13b)

Following the general procedure, method 1, the title compound was obtained by treatment of **11** with ethylamine in MeOH. Yield: 76%, mp 129 °C. ^{1}H NMR ($CDCl_3$): δ 1.22 (t, J = 7.2 Hz, 3H), 3.27 (q, J = 7.2 Hz, 2H), 6.79 (dd, J = 2.4 Hz and 8.4 Hz, 1H), 7.05 (d, J = 2.4 Hz, 1H), 7.33 (d, J = 8.4 Hz, 1H). MS (EI): m/z 231 $[M]^+$.

4.2.3. *N*-(Isopropyl)-*N*′-(3,4-dichlorophenyl)-guanidine (13c)

Following the general procedure, method 1, the title compound was obtained by treatment of **11** with isopropylamine. Yield: 92%, mp 179 °C. ^{1}H NMR (acetone-d_6): δ 1.35 (d, J = 6.3 Hz, 6H), 2.89 (br s, 3H), 4.18 (m, 1H), 7.41 (dd, J = 2.4 Hz, and 8.7 Hz, 1H), 7.64–7.68 (m, 2H). MS (EI): m/z 245 $[M]^+$.

4.2.4. *N*-(*t*-Butyl)-*N*′-(3,4-dichlorophenyl)-guanidine (13d)

Following the general procedure, method 1, the title compound was obtained by treatment of **11** with *tert*-butylamine. Yield: 74%, mp 149 °C. ^{1}H NMR ($CDCl_3$): δ 1.40 (s, 9H), 3.09 (br s, 3H), 6.75 (dd, J = 2.4 Hz, and 8.4 Hz, 1H), 7.01 (d, J = 2.4 Hz, 1H), 7.31 (d, J = 8.4 Hz, 1H). MS (EI): m/z 259 $[M]^+$.

4.2.5. *N*-Neopenty-*N*′-(3,4-dichlorophenyl)-guanidine (13e)

Following the general procedure, method 1, the title compound was obtained by treatment of **11** with neopentylamine. Yield: 78%, mp 107 °C. ^{1}H NMR ($CDCl_3$): δ 0.95 (s, 9H), 3.00 (s, 2H), 4.11 (br s, 3H), 6.76 (br d, J = 8.4 Hz, 1H), 7.02 (s, 1H), 7.31 (br d, J = 8.4 Hz, 1H). MS (EI): m/z 274 $[M]^+$.

4.2.6. *N*-Phenyl-*N*′-(3,4-dichlorophenyl)-guanidine (13f)

Following the general procedure, method 2, the title compound was obtained by treatment of **14** with 2 equiv of aniline HCl. Yield: 73%, mp. 192 °C. ^{1}H NMR (CD_3OD): δ 6.98–7.03 (m, 2H), 7.21–7.37 (m, 6H). MS (EI): m/z 279 $[M]^+$.

4.2.7. *N*-Benzyl-*N*′-(3,4-dichlorophenyl)-guanidine (13g)

Following the general procedure, Method 1, the title compound was obtained by treatment of **11** with benzylamine. Yield: 88%, mp 126 °C. ^{1}H NMR ($CDCl_3$): δ 4.40 (s, 2H), 6.76 (dd, J = 2.4 Hz, and 8.5 Hz, 1H), 7.03 (d, J = 2.5 Hz, 1H), 7.26–7.37 (m, 6H). MS (EI): m/z 293 $[M]^+$.

4.2.8. *N*-(*n*-Hexyl)-*N*′-(3,4-dichlorophenyl)-guanidine (13h)

Following the general procedure, method 1, the title compound was obtained by treatment of **11** with *n*-hexylamine. Yield: 95%, oil. ^{1}H NMR ($CDCl_3$): δ 0.89 (t, J = 6.5 Hz, 3H), 1.30 (br s, 6H), 1.52 (br s, 2H), 3.11–3.20 (m, 2H), 6.77 (dd, J = 2.7 Hz, and 8.5 Hz, 1H), 7.02 (d, J = 2.7 Hz, 1H), 7.30 (d, J = 8.5 Hz, 1H). MS (EI): m/z 287 $[M]^+$.

4.2.9. *N*-(2-Adamantyl)-*N*′-(3,4-dichlorophenyl)-guanidine (13i)

Following the general procedure, method 1, the title compound was obtained by treatment of **11** with 2-adamantylamine HCl. Yield: 55%, mp 212 °C. ^{1}H NMR ($CDCl_3$): δ 1.62–2.05 (m, 15H), 3.73 (s, 1H), 6.79 (dd, J = 2.4 Hz, and 8.5 Hz, 1H), 7.06 (d, J = 2.4 Hz, 1H), 7.32 (d, J = 8.5 Hz, 1H). MS (EI): m/z 337 $[M]^+$.

4.2.10. *N*-(2-Dimethylaminoethyl)-*N*′-(3,4-dichlorophenyl)-guanidine (13j)

Following the general procedure, method 2, the title compound was obtained by treatment of **14** with *N*,*N*-dimethylethylenediamine. Yield: 61%, brown oil. ^{1}H NMR ($CDCl_3$): δ 2.25 (s, 6H), 2.46 (t, J = 5.2 Hz, 2H), 3.25 (t, J = 5.2 Hz, 2H), 4.74 (br s, 1H), 6.76 (dd, J = 2.40 Hz, and 8.49 Hz, 1H), 7.03 (d, J = 2.4 Hz, 1H), 7.29 (d, J = 8.5 Hz, 1H). MS (EI): m/z 275 $[M]^+$.

4.2.11. *N*-(2-Diethylaminoethyl)-*N*′-(3,4-dichlorophenyl)-guanidine (13k)

Following the general procedure, method 2, the title compound was obtained by treatment of **14** with *N*,*N*-diethylethylenediamine. Yield: 77%, yellow oil. ^{1}H NMR ($CDCl_3$): δ 1.00 (t, J = 7.2 Hz, 6H), 2.50 (m, 6H), 3.20 (t, J = 4.0 Hz, 2H), 5.17 (br s, 1H), 6.75 (dd, J = 2.0 Hz, and 8.5 Hz, 1H), 7.01 (d, J = 2.0 Hz, 1H), 7.27 (d, J = 8.5 Hz, 1H). MS (EI): m/z 303 $[M]^+$.

4.2.12. *N*-(2-Pyrrolinoethyl)-*N*′-(3,4-dichlorophenyl)-guanidine (13l)

Following the general procedure, method 2, the title compound was obtained by treatment of **14** with 1-(2-aminethyl)pyrrolidine. Yield: 46%, mp 146 °C. ^{1}H NMR ($CDCl_3$): δ 1.77 (br s, 4H), 2.56 (br s, 4H), 2.66 (t, J = 5.2 Hz, 2H), 3.28 (t, J = 5.2 Hz, 2H), 6.77 (dd, J = 2.2 Hz, and 8.4 Hz, 1H), 7.03 (d, J = 2.2 Hz, 1H), 7.34 (d, J = 8.4 Hz, 1H). MS (EI): m/z 301 $[M]^+$.

4.2.13. *N*-(3-Dimethylaminopropyl)-*N*′-(3,4-dichlorophenyl)-guanidine (13m)

Following the general procedure, method 2, the title compound was obtained by treatment of **14** with 3-(dimethylamino)-1-propylamine. Yield: 52%, mp 90 °C. ^{1}H NMR ($CDCl_3$): δ 1.65 (br s, 2H), 2.16 (s, 6H), 2.37 (t, J = 5.5 Hz, 2H), 3.26 (t, J = 5.5 Hz, 2H), 6.77 (br d, J = 8.1 Hz, 1H), 7.03 (br s, 1H), 7.29 (br d, J = 8.1 Hz, 1H). MS (EI): m/z 289 $[M]^+$.

4.2.14. *N*-(4-Pyrrolinobutyl)-*N*′-(3,4-dichlorophenyl)-guanidine (13n)

Following the general procedure, method 2, the title compound was obtained by treatment of **14** with 4-(1-pyrrolidino)-butylamine. Yield: 68%, brown oil. ^{1}H NMR ($CDCl_3$): δ 1.60 (m, 4H), 1.76 (br s, 4H), 2.48 (br s, 6H), 3.21 (t, J = 6.6 Hz, 2H), 4.05 (br s, 2H), 6.75 (dd, J = 2.3 Hz, and 8.5 Hz, 1H), 7.02 (d, J = 2.3 Hz, 1H), 7.28 (d, J = 8.5 Hz, 1H). MS (EI): m/z 329 $[M]^+$.

4.2.15. *N*-(6-*t*-Butoxycarbonylaminohexyl)-*N*′-(3,4-dichlorophenyl)-guanidine (13p)

Following the general procedure, method 2, the title compound was obtained by treatment of **14** with *N*-Boc-1,6-hexanediamine. Yield: 67%, brown oil. ^{1}H NMR ($CDCl_3$): δ 1.30–1.60 (m, 8H), 1.44 (s, 9H), 3.09–3.16 (m, 2H), 3.21 (t, J = 6.9 Hz, 2H), 6.77 (dd, J = 2.4 Hz, and 8.5 Hz, 1H), 7.03 (d, J = 2.4 Hz, 1H), 7.30 (d, J = 8.4 Hz, 1H). MS (EI): m/z 402 $[M]^+$.

4.3. General procedure for the synthesis of *N*-alkoxy (8a–e) and *N*-alkyl (9a–p) derivatives of *N*′-(3,4-dichlorophenyl)-*N*″-(1-isopropyl-4,5-dioxoimidazolidine-2-ylidene)guanidine (1)

A mixture of guanidine 12 or 13 (1.0 equiv) and 1-isopropyl-2-methylsulfanyl-1*H*-imidazole-4,5-dione (10, 1.1–1.5 equiv) in anhydrous $CHCl_3$ was heated in a sealed tube at 100 °C for 18–72 h. The solvent was removed under reduced pressure. The residue was purified via a silica gel column chromatography. Chloroform and $CHCl_3$/EtOAc were used as eluent for the purification of **8a**–**e** and **9a**–**p**, respectively. The former was further purified by recrystallization from CH_2Cl_2/hexane and the latter was purified by recrystallization from EtOAc.

4.3.1. *N*-(3,4-Dichloro-phenyl)-*N*′-(1-isopropyl-4,5-dioxo-4,5-dihydro-1*H*-imidazol-2-yl)-*N*″-methoxyguanidine (8a)

Following the general procedure, the title compound was obtained from **12a**. Yield: 20%; mp 156 °C. ^{1}H NMR ($CDCl_3$): δ 1.45 (d, J = 7.0 Hz, 6H), 3.88 (s, 3H), 4.55 (m, 1H), 6.94 (dd, J = 2.5 Hz, and 8.6 Hz, 1H), 7.12 (br s, 1H), 7.35 (d, J = 8.6 Hz, 1H), 7.46 (s, 2.5 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 19.5, 45.8, 62.3, 121.1, 124.1,

127.1, 130.2, 132.4, 137.6, 145.5, 149.6, 155.8, 155.9; MS (EI): *m/z* 371 $[M]^+$. Anal. Calcd for $C_{14}H_{15}Cl_2N_5O_3$: C, 45.18; H, 4.06; N, 18.82; Cl, 19.05. Found: C, 45.46; H, 4.10; N, 18.80; Cl, 19.21.

4.3.2. *N*-(3,4-Dichloro-phenyl)-*N*′-(1-isopropyl-4,5-dioxo-4,5-dihydro-1*H*-imidazol-2-yl)-*N*″-ethoxyguanidine (8b)

Following the general procedure, the title compound was obtained from **12b**. Yield: 48%; mp 155 °C. ^{1}H NMR ($CDCl_3$): δ 1.31 (t, J = 7.0 Hz, 3H), 1.45 (d, J = 7.0 Hz, 6H), 4.10 (q, J = 7.0 Hz, 2H), 4.54 (m, 1H), 6.95 (dd, J = 8.7, and 2.6 Hz, 1H), 7.13 (br s, 1H), 7.34 (d, J = 8.7 Hz, 1H), 7.46 (d, J = 2.5 Hz, 1H), 10.52 (br s, 1H); ^{13}C NMR ($CDCl_3$): δ 14.6, 19.5, 45.8, 70.1, 121.2, 124.1, 127.0, 130.2, 132.4, 137.7, 145.4, 149.5, 155.8, 156.9; MS (EI): *m/z* 385 $[M]^+$. Anal. Calcd for $C_{15}H_{17}Cl_2N_5O_3$: C, 46.65; H, 4.44; N, 18.36; Cl, 18.13. Found: C, 46.49; H, 4.35; N, 18.54; Cl, 17.83.

4.3.3. *N*-(3,4-Dichloro-phenyl)-*N*′-(1-isopropyl-4,5-dioxo-4,5-dihydro-1*H*-imidazol-2-yl)-*N*″-allyloxyguanidine (8c)

Following the general procedure, the title compound was obtained from **12c**. Yield: 10%; mp 266 °C. ^{1}H NMR ($CDCl_3$): δ 1.45 (d, J = 7.0 Hz, 6H), 4.52–4.57 (m, 3H), 5.32–5.41 (m, 2H), 5.96–6.09 (m, 1H), 6.95 (dd, J = 2.5 Hz, and 8.6 Hz, 1H), 7.14 (br s, 1H), 7.33 (d, J = 8.6 Hz, 1H), 7.47 (d, J = 2.5 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 19.5, 45.8, 75.3, 77.2, 118.8, 121.2, 124.1, 127.1, 130.2, 132.4, 134.0, 137.6, 145.7, 149.7, 155.7, 155.9; MS (EI) *m/z* 397 $[M]^+$. Anal. Calcd for $C_{16}H_{17}Cl_2N_5O_3$: C, 48.25; H, 4.30; N, 17.59; Cl, 17.80. Found: C, 48.31; H, 4.36; N, 17.55; Cl, 18.03.

4.3.4. *N*-(3,4-Dichloro-phenyl)-*N*′-(1-isopropyl-4,5-dioxo-4,5-dihydro-1*H*-imidazol-2-yl)-*N*″-*t*-butoxyguanidine (8d)

Following the general procedure, the title compound was obtained from **12d**. Yield: 70%; mp 250 °C. ^{1}H NMR ($CDCl_3$): δ 1.36 (s, 9H), 1.46 (d, J = 7.0 Hz, 6H), 4.56 (m, 1H), 6.97 (dd, J = 2.5 Hz, and 8.6 Hz, 1H), 7.15 (br s, 1H), 7.34 (d, J = 8.6 Hz, 1H), 7.47 (d, J = 2.5 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 19.5, 27.7, 45.7, 79.4, 121.1, 124.0, 126.7, 130.1, 132.3, 137.9, 145.3, 149.1, 155.8, 156.0; MS (EI): *m/z* 413 $[M]^+$. Anal. Calcd for $C_{17}H_{21}Cl_2N_5O_3$: C, 49.29; H, 5.11; N, 16.90; Cl, 17.12. Found: C, 49.14; H, 5.14; N, 16.67; Cl, 17.32.

4.3.5. *N*-(3,4-Dichloro-phenyl)-*N*′-(1-isopropyl-4,5-dioxo-4,5-dihydro-1*H*-imidazol-2-yl)-*N*″-benzyloxyguanidine (8e)

Following the general procedure, the title compound was obtained from **12e**. Yield: 20%; mp 174 °C. ^{1}H NMR ($CDCl_3$): δ 1.43 (d, J = 7.0 Hz, 6H), 4.50 (m, 1H), 5.05 (s, 2H), 6.93 (dd, J = 2.7 Hz, and 8.6 Hz, 1H), 7.14 (br s, 1H), 7.33 (d, J = 8.6 Hz, 1H), 7.39–7.44 (m, 5H); ^{13}C NMR ($CDCl_3$): δ 19.7, 46.0, 76.8, 121.4, 124.3, 124.4, 127.4, 128.8, 129.0, 130.4, 132.6, 137.2, 137.7, 146.0, 150.0, 155.8, 156.2; MS (EI): *m/z* 447 $[M]^+$. Anal. Calcd for $C_{20}H_{19}Cl_2N_5O_3$: C, 53.58; H, 4.27; N, 15.62; Cl, 15.82. Found: C, 53.47; H, 4.23; N, 15.55; Cl, 15.68.

4.3.6. *N*-(3,4-Dichloro-phenyl)-*N*′-(1-isopropyl-4,5-dioxo-4,5-dihydro-1*H*-imidazol-2-yl)-*N*″-methylguanidine (9a)

Following the general procedure, the title compound was obtained from **13a**. Yield: 40%; mp 229 °C. ^{1}H NMR ($CDCl_3$): δ 1.48 (d, J = 6.9 Hz, 6H), 3.10 (s, 3H), 4.58 (m, 1H), 7.14 (br d, J = 8.6 Hz, 1H), 7.40 (s, 1H), 7.56 (d, J = 8.6 Hz, 1H); ^{13}C NMR (DMSO-d_6): δ 170.29, 168.24, 161.73, 159.02, 137.87, 136.26, 131.82, 130.67, 127.75, 125.68, 43.89, 29.51, 20.04; MS (EI): *m/z* 355 $[M]^+$. Anal. Calcd for $C_{14}H_{15}Cl_2N_5O_2$: C, 47.21; H, 4.24; N, 19.91; Cl, 19.66. Found: C, 47.28; H, 4.22; N, 20.07; Cl, 19.52.

4.3.7. *N*-(3,4-Dichloro-phenyl)-*N*′-(1-isopropyl-4,5-dioxo-4,5-dihydro-1*H*-imidazol-2-yl)-*N*″-ethylguanidine (9b)

Following the general procedure, the title compound was obtained from **13b**. Yield: 26%; mp 217 °C. ^{1}H NMR ($CDCl_3$): δ 1.24 (t, J = 7.0 Hz, 3H), 1.47 (d, J = 6.9 Hz, 6H), 3.57 (q, J = 7.4 Hz, 2H), 4.55 (m, 1H), 7.12 (d, J = 8.74, Hz, 1H), 7.39 (br s, 1H), 7.55 (d, J = 8.6 Hz, 1H), 10.5 (br s, 1H); ^{13}C NMR (DMSO-d_6): δ 13.6, 19.2, 39.0, 47.4, 119.9, 124.2, 127.0, 131.3, 131.8, 155.6, 158.3; MS (EI): *m/z* 369 $[M]^+$. Anal. Calcd for $C_{15}H_{17}Cl_2N_5O_2$: C, 48.66; H, 4.63; N, 19.15; Cl, 18.92. Found: C, 48.48; H, 4.47; N, 19.25; Cl, 18.72.

4.3.8. *N*-(3,4-Dichloro-phenyl)-*N*′-(1-isopropyl-4,5-dioxo-4,5-dihydro-1*H*-imidazol-2-yl)-*N*″-isopropylguanidine (9c)

Following the general procedure, the title compound was obtained from **13c**. Yield: 20%; mp 191 °C. ^{1}H NMR ($CDCl_3$): δ 1.27 (d, J = 6.6 Hz, 6H), 1.47 (d, J = 6.9 Hz, 6H), 4.39 (m, 1H), 4.53 (m, 1H), 7.11 (dd, J = 2.5 Hz, and 8.4 Hz, 1H), 7.38 (d, J = 2.5 Hz, 1H), 7.57 (d, J = 8.4 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 20.2, 22.7, 44.6, 45.1, 125.1, 127.8, 132.0, 132.4, 134.1, 134.3, 157.6, 161.1, 169.9, 171.9; MS (EI): *m/z* 383 $[M]^+$. Anal. Calcd for $C_{16}H_{19}Cl_2N_5O_2$: C, 50.01; H, 4.98; N, 18.45; Cl, 18.23. Found: C, 49.86; H, 5.05; N, 17.99; Cl, 18.58.

4.3.9. *N*-(3,4-Dichloro-phenyl)-*N*′-(1-isopropyl-4,5-dioxo-4,5-dihydro-1*H*-imidazol-2-yl)-*N*″-*t*-butylguanidine (9d)

Following the general procedure, the title compound was obtained from **13d**. Yield: 26%; mp 243 °C. ^{1}H NMR ($CDCl_3$): δ 1.42 (s, 9H), 1.51 (d, J = 6.9 Hz, 6H), 4.57 (m, 1H), 5.19 (br s, 1H), 7.10 (dd, J = 2.2 Hz, and 8.4 Hz, 1H), 7.37 (br s, 1H), 7.56 (d, J = 8.5 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 19.8, 29.6, 44.9, 54.1, 124.8, 127.6, 131.9, 134.3, 158.1, 161.2, 169.9, 171.2; MS (EI): *m/z* 397 $[M]^+$. Anal. Calcd for $C_{17}H_{21}Cl_2N_5O_2$: C, 51.27; H, 5.31; N, 17.80; Cl, 17.58. Found: C, 51.30; H, 5.32; N, 17.99; Cl, 17.29.

4.3.10. *N*-(3,4-Dichloro-phenyl)-*N*′-(2,2-dimethyl-propyl)-*N*″-(1-isopropyl-4,5-dioxo-4,5-dihydro-1*H*-imidazol-2-yl)-guanidine (9e)

Following the general procedure, the title compound was obtained from **13e**. Yield: 37%; mp 203 °C. Major tautomer: ^{1}H NMR ($CDCl_3$): δ 0.94 (s, 9H), 1.48 (d, J = 6.5 Hz, 6H), 3.38 (br d, J = 5.8 Hz, 2H), 4.55 (m, 1H), 7.14 (br d, J = 8.3 Hz, 1H), 7.41 (br s, 1H), 7.58 (br d, J = 8.3 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 20.1, 27.4, 31.7, 44.7, 54.1, 124.4, 127.5, 127.5, 129.9, 131.9, 136.4, 157.8, 161.6, 170.7, 171.5; Minor tautomer: ^{1}H NMR ($CDCl_3$) δ 1.07 (s, 9H), 1.30 (d, J = 7.1 Hz, 6H), 3.23 (br s, 2H), 4.34 (m, 1H), 7.20 (br d, J = 8.3 Hz, 1H), 7.55 (br s, 1H), 7.64 (br s, 1H); ^{13}C NMR ($CDCl_3$): δ.19.8, 27.4, 32.6, 44.6, 53.3, 125.1, 127.8, 129.4, 131.8, 134.1, 159.2, 161.5, 170.3, 171.5. MS (EI): *m/z* 411 $[M]^+$. Anal. Calcd for $C_{18}H_{23}Cl_2N_5O_2$: C, 52.43; H, 5.62; N, 17.20; Cl, 16.99. Found: C, 52.66; H, 5.71; N, 16.91; Cl, 16.81.

4.3.11. *N*-(3,4-Dichloro-phenyl)-*N*′-(1-isopropyl-4,5-dioxo-4,5-dihydro-1*H*-imidazol-2-yl)-*N*″-phenylguanidine (9f)

Following the general procedure, the title compound was obtained from **13f**. Yield: 25%; mp 189 °C. ^{1}H NMR ($CDCl_3$): δ 1.34 (d, J = 6.9 Hz, 6H), 4.40 (m, 1H), 7.10 (br d, J = 8.1 Hz, 1H), 7.32–7.60 (m, 7H); ^{13}C NMR ($CDCl_3$): δ 19.8, 44.7, 123.8, 125.6, 127.1, 128.2, 130.4, 132.6, 134.2, 135.0, 157.1, 161.0, 169.8, 172.4; MS (EI): *m/z* 417 $[M]^+$; Anal: Calcd for $C_{19}H_{17}Cl_2N_5O_2$: C, 54.56; H, 4.10; N, 16.74; Cl, 16.95. Found: C, 54.39; H, 3.98; N, 16.60; Cl, 16.90.

4.3.12. *N*-(3,4-Dichloro-phenyl)-*N*′-(1-isopropyl-4,5-dioxo-4,5-dihydro-1*H*-imidazol-2-yl)-*N*″-benzylguanidine (9g)

Following the general procedure, the title compound was obtained from **13g**. Yield: 34%; mp 190 °C. ^{1}H NMR ($CDCl_3$): δ 1.40 (d, J = 6.8 Hz, 6H), 4.52 (m, 1H), 4.74 (br d, J = 5.4 Hz, 2H), 5.97 (br s, 1H), 7.13 (br d, J = 8.5 Hz, 1H), 7.25–7.40 (m, 6H), 7.53 (d, J = 8.5 Hz, 1H); Major tautomer: ^{13}C NMR ($CDCl_3$) δ 19.6, 43.5, 45.5, 125.2, 127.5, 127.8, 128.4, 129.4, 130.3, 131.5, 136.1, 138.1, 159.0, 161.3, 169.5; Minor tautomer: ^{13}C NMR ($CDCl_3$) δ 19.6,

43.5, 45.3, 125.8, 127.1, 127.8, 128.4, 129.4, 130.6, 131.8, 136.1, 137.2, 155.5, 161.2, 168.9; MS (EI): *m/z* 431 $[M]^+$. Anal. Calcd for $C_{20}H_{19}Cl_2N_5O_2$: C, 55.57; H, 4.43; N, 16.40; Cl, 16.20. Found: C, 55.38; H, 4.43; N, 16.52; Cl, 16.01.

4.3.13. *N*-(3,4-Dichloro-phenyl)-*N*′-(1-isopropyl-4,5-dioxo-4,5-dihydro-1*H*-imidazol-2-yl)-*N*″-*N*-*hexyl*-*guanidine* (9h)

Following the general procedure, the title compound was obtained from **13h**. Yield: 21%; mp 147 °C. ^{1}H NMR ($CDCl_3$): δ 0.86–0.91 (m, 3H), 1.30 (br s, 6H), 1.47 (d, J = 6.9 Hz, 6H), 1.58–1.63 (m, 2H), 3.49–3.54 (m, 2H), 4.55 (m, 1H), 7.12 (dd, J = 2.2 Hz, and 8.5 Hz, 1H), 7.39 (d, J = 2.2 Hz, 1H), 7.54 (d, J = 8.5 Hz, 1H); Major tautomer: ^{13}C NMR ($CDCl_3$) δ 14.0, 20.1, 22.5, 26.4, 29.6, 31.4, 42.7, 44.7, 124.5, 127.6, 129.9, 131.6, 133.8, 136.4, 157.4, 161.5, 170.4, 170.9; Minor tautomer: ^{13}C NMR ($CDCl_3$) δ 14.0, 19.8, 22.5, 26.5, 28.6, 31.3, 42.5, 44.7, 125.1, 127.6, 129.4, 131.9, 134.3, 136.4, 158.6, 161.5, 170.4, 171.4; LC–MS (ESI): *m/z* 426 $[M+1]^+$. Anal. Calcd for $C_{19}H_{25}Cl_2N_5O_2$: C, 53.53; H, 5.91; N, 16.43; Cl, 16.63. Found: C, 53.47; H, 5.84; N, 16.27; Cl, 16.74.

4.3.14. *N*-(3,4-Dichloro-phenyl)-*N*′-(1-isopropyl-4,5-dioxo-4,5-dihydro-1*H*-imidazol-2-yl)-*N*″-(2-adamantyl)-guanidine (9i)

Following the general procedure, the title compound was obtained from **13i**. Yield: 32%; mp 251 °C. ^{1}H NMR (DMSO-d_6 with D_2O): δ 1.17 (d, J = 6.7 Hz, 6H), 1.64–1.74 (m, 4H), 1.87–2.03 (m, 10H), 4.07 (s, 1H), 4.17 (m, 1H), 7.34 (br d, J = 8.6 Hz, 1H), 7.66 (br d, J = 8.6 Hz, 1H), 7.74 (s, 1H); Major tautomer: ^{13}C NMR ($CDCl_3$) δ 20.3, 26.7, 31.9, 36.8, 37.0, 44.6, 56.5, 124.4, 127.3, 129.9, 132.2, 134.0, 136.3, 157.3, 161.1, 169.9, 171.6; Minor tautomer: ^{13}C NMR ($CDCl_3$) δ 19.8, 26.6, 31.9, 36.6, 37.1, 44.5, 56.8, 124.5, 127.7, 129.5, 132.5, 134.6, 136.3, 156.1, 161.5, 170.7, 172.2; MS (EI): *m/z* 475 $[M]^+$. Anal. Calcd for $C_{23}H_{27}Cl_2N_5O_2$: C, 57.99; H, 5.71; N, 14.70; Cl, 14.88. Found: C, 57.86; H, 5.66; N, 14.62; Cl, 14.80.

4.3.15. *N*-(3,4-Dichloro-phenyl)-*N*′-(2-dimethylamino-ethyl)-*N*″-(1-isopropyl-4,5-dioxo-4,5-dihydro-1*H*-imidazol-2-yl)-guanidine (9j)

Following the general procedure, the title compound was obtained from **13j**. Yield: 18%; mp 198 °C. ^{1}H NMR ($CDCl_3$): δ 1.34 (d, J = 6.9 Hz, 6H), 2.46 (s, 6H), 2.78 (t, J = 4.1 Hz, 2H), 3.51 (br s, 2H), 4.39 (m, 1H), 6.95 (dd, J = 2.4 Hz, 8.6 Hz, 1H), 7.40 (d, J = 8.6 Hz, 1H), 7.58 (d, J = 2.4 Hz, 1H), 10.61 (br s, 1H); ^{13}C NMR ($CDCl_3$): δ 19.9, 41.7, 44.6, 45.0, 60.6, 121.9, 125.4, 128.4, 130.3, 132.6, 137.5, 160.0, 161.3, 170.6, 171.7; LC–MS (ESI): *m/z* 413 $[M+1]^+$. Anal. Calcd for $C_{17}H_{22}Cl_2N_6O_2$: C, 49.40; H, 5.37; N, 17.16; Cl, 20.33. Found: C, 49.60; H, 5.53; N, 17.03; Cl, 19.97.

4.3.16. *N*-(3,4-Dichloro-phenyl)-*N*′-(2-diethylamino-ethyl)-*N*″-(1-isopropyl-4,5-dioxo-4,5-dihydro-1*H*-imidazol-2-yl)-guanidine (9k)

Following the general procedure, the title compound was obtained from **13k**. Yield: 17%; mp 139 °C. ^{1}H NMR ($CDCl_3$): δ 1.10 (t, J = 7.2 Hz, 6H), 1.32 (d, J = 7.0 Hz, 6H), 2.73 (q, J = 7.8 Hz, 4H), 2.78 (t, J = 3.9 Hz, 2H), 3.52 (br s, 2H), 4.36 (m, 1H), 6.97 (dd, J = 2.4 Hz, and 8.6 Hz, 1H), 7.40 (d, J = 8.6 Hz, 1H), 7.52 (d, J = 2.4 Hz, 1H), 10.60 (br s, 1H); ^{13}C NMR ($CDCl_3$): δ 11.0, 19.9, 42.7, 44.5, 47.2, 55.2, 122.5, 126.1, 128.8, 130.3, 132.6, 137.1, 160.4, 161.3, 170.6, 171.9; LC–MS (ESI): *m/z* 441 $[M+1]^+$. Anal. Calcd for $C_{19}H_{26}Cl_2N_6O_2$: C, 51.71; H, 5.94; N, 16.07; Cl, 19.04. Found: C, 51.69; H, 5.95; N, 16.19; Cl, 18.91.

4.3.17. *N*-(3,4-Dichloro-phenyl)-*N*′-(1-isopropyl-4,5-dioxo-4,5-dihydro-1*H*-imidazol-2-yl)-*N*″-(2-pyrrolidin-1-yl-ethyl)-guanidine (9l)

Following the general procedure, the title compound was obtained from **13l**. Yield: 16%; mp 196 °C. ^{1}H NMR ($CDCl_3$): δ 1.33 (d, J = 6.9 Hz, 6H), 1.91 (br s, 4H), 2.85 (br s, 4H), 2.92–2.95 (m, 2H), 3.55 (br s, 2H), 4.38 (m, 1H), 6.98 (d, J = 8.6 Hz, 1H), 7.41 (d, J = 8.6 Hz, 1H), 7.49 (s, 1H); ^{13}C NMR ($CDCl_3$): δ 19.7, 23.6, 41.9, 44.3, 53.6, 57.0, 122.3, 125.7, 128.1, 130.1, 132.2, 138.2, 159.6, 161.3, 170.4; LC–MS (ESI): *m/z* 439 $[M+1]^+$. Anal. Calcd for $C_{19}H_{24}Cl_2N_6O_2$: C, 51.94; H, 5.51; N, 19.13; Cl, 16.14. Found: C, 51.78; H, 5.59; N, 18.84; Cl, 15.89.

4.3.18. *N*-(3,4-Dichloro-phenyl)-*N*′-(3-dimethylamino-propyl)-*N*″-(1-isopropyl-4,5-dioxo-4,5-dihydro-1*H*-imidazol-2-yl)-guanidine (9m)

Following the general procedure, the title compound was obtained from **13m**. Yield: 31%; mp 110 °C. Major tautomer: ^{1}H NMR ($CDCl_3$) δ 1.33 (d, J = 6.9 Hz, 6H), 1.70 (br s, 2H), 1.89 (s, 6H), 2.41–2.44 (m, 2H), 3.64 (br s, 2H), 4.37 (m, 1H), 7.06 (dd, J = 2.4 Hz, and 8.6 Hz, 1H), 7.40 (d, J = 2.6 Hz, 1H), 7.42 (d, J = 8.6 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 20.8, 27.9, 40.8, 45.4, 45.6, 54.7, 123.9, 127.2, 131.2, 132.4, 133.5, 138.6, 160.8, 162.2, 171.6, 172.7; Minor tautomer: ^{1}H NMR ($CDCl_3$) δ 1.47 (d, J = 6.9 Hz, 6H), 1.89 (br s, 2H), 2.22 (s, 6H), 2.47–2.52 (m, 2H), 3.66–3.68 (m, 2H), 4.56 (m, 1H), 7.13 (dd, J = 2.6 Hz, and 8.5 Hz, 1H), 7.54 (d, J = 8.5 Hz, 1H), 7.64 (d, J = 2.8 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 21.1, 24.4, 40.8, 45.3, 45.8, 60.6, 126.9, 129.3, 129.8, 132.8, 134.6, 136.0, 159.2, 162.2, 171.0, 172.5; LC–MS (ESI): *m/z* 427 $[M+1]^+$. Anal. Calcd for $C_{18}H_{24}Cl_2N_6O_2$: C, 50.59; H, 5.66; N, 19.67; Cl, 16.59. Found: C, 50.46; H, 5.66; N, 19.76; Cl, 16.48.

4.3.19. *N*-(3,4-Dichloro-phenyl)-*N*′-(1-isopropyl-4,5-dioxo-4,5-dihydro-1*H*-imidazol-2-yl)-*N*″-(4-pyrrolidin-1-yl-butyl)-guanidine (9n)

Following the general procedure, the title compound was obtained from **13n**. Yield: 23%; mp 80 °C. Major tautomer: ^{1}H NMR ($CDCl_3$): δ 1.44 (d, J = 6.9 Hz, 6H), 1.83 (br s, 4H), 2.18 (br s, 4H), 2.98 (br s, 2H), 3.18 (br s, 2H), 3.37 (br s, 1H), 3.52 (br s, 1H), 3.78 (br s, 2H), 4.56 (m, 1H), 7.05 (dd, J = 2.4 and 8.6 Hz, 1H), 7.36 (d, J = 2.4 Hz, 1H), 7.46 (d, J = 8.6 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 20.1, 23.3, 26.3, 27.6, 42.7, 44.5, 54.1, 55.8, 124.0, 126.3, 131.4, 133.6, 135.4, 139.3, 158.5, 161.5, 168.6, 170.5; Minor tautomer: ^{1}H NMR ($CDCl_3$) δ 1.32 (d, J = 6.9 Hz, 6H), 1.74 (br s, 4H), 2.15 (br s, 4H), 2.99 (br s, 2H), 3.17 (br s, 2H), 3.39 (br s, 1H), 3.54 (br s, 1H), 3.79 (br s, 2H), 4.42 (m, 1H), 7.07 (dd, J = 2.4 and 8.5 Hz, 1H), 7.32 (d, J = 2.4 Hz, 1H), 7.49 (d, J = 8.5 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 19.8, 24.0, 29.6, 31.6, 40.5, 43.7, 55.2, 60.4, 125.3, 128.1, 129.1, 129.9, 130.7, 132.1, 158.1, 161.5, 168.5, 171.1; LC–MS (ESI): *m/z* 467 $[M+1]^+$. Anal. Calcd for $C_{21}H_{28}Cl_2N_6O_2 \cdot 0.1H_2O$: C, 53.76; H, 6.06; N, 15.11; Cl, 17.91. Found: C, 53.43; H, 6.03; N, 14.93; Cl, 17.80.

4.3.20. {6-[*N*′-(3,4-Dichloro-phenyl)-*N*″-(1-isopropyl-4,5-dioxo-4,5-dihydro-1*H*-imidazol-2-yl)-guanidino]-hexyl}-carbamic acid *tert*-butyl ester (9p)

Following the general procedure, the title compound was obtained from **13p**. Yield: 39%, mp 142 °C. ^{1}H NMR ($CDCl_3$): δ 1.30–1.80 (m, 8H), 1.36 (s, 9H), 1.47 (d, J = 6.9 Hz, 6H), 3.06–3.19 (m, 2H), 3.43–3.55 (m, 2H), 4.55 (m, 1H), 7.13 (dd, J = 2.2 Hz, and 8.5 Hz, 1H), 7.41 (d, J = 2.2 Hz, 1H), 7.52 (d, J = 8.5 Hz, 1H); Major tautomer: ^{13}C NMR ($CDCl_3$) δ 20.0, 25.5, 26.0, 28.2, 29.3, 29.9, 39.6, 42.2, 44.5, 79.0, 124.4, 127.5, 129.8, 131.4, 133.6, 136.3, 156.0, 161.4, 170.7, 171.2; Minor tautomer: ^{13}C NMR ($CDCl_3$) δ 19.7, 25.4, 26.3, 28.3, 29.7, 29.9, 40.2, 41.8, 44.5, 125.2, 127.7, 129.3, 131.8, 134.2, 136.3, 157.4, 158.3, 170.2, 171.2; LC–MS (ESI): *m/z* 541 $[M+1]^+$. Anal. Calcd for $C_{24}H_{34}Cl_2N_6O_4$: C, 53.24; H, 6.33; N, 15.52; Cl, 13.10. Found: C, 53.23; H, 6.40; N, 15.47; Cl, 13.05.

4.4. Biological studies

4.4.1. Assessment of metabolic stability

The in vitro half-life of each compound was measured in the presence of human and mouse liver microsomes. The metabolic stability assay sample preparation was performed in a 96-well plate on a TECAN Genesis robotic sample processor following WRAIR SOP SP 01-02. Samples were analyzed by LC–MS/MS using fast LC gradient or isocratic methods. Parent drug was quantified using external calibration, using plots of parent drug response versus amount. Chromatograms were analyzed using the mass spectrometry software Xcalibur QuanBrowser (for ThermoFinnigan instruments) or MassLynx (for Waters instruments). Concentrations of parent drug remaining at each time point were calculated using the unknown peak areas and corresponding calibration curves. In order to calculate the half-life, a first-order rate of decay was assumed. A plot of the natural log (LN) of the drug concentration versus time was generated, where the slope of that line was $-k$. Half-life was calculated as $0.693/k$. The results are shown in Table 1.

4.4.2. In vitro antimalarial studies

The in vitro assays were conducted by using a modification of the semi automated microdilution technique of Desjardins et al and Chulay et al.[14,15] Two *P. falciparum* malaria parasite clones, from CDC Indochina III (W-2), and CDC Sierra Leone I (D-6), were utilized in susceptibility testing. They were derived by direct visualization and micromanipulation from patient isolates.[16] The results are shown in Table 1.

4.4.3. Assessment of prophylactic activity in mice

New compounds were assessed for their prophylactic activity in exoerythrocytic (EE) mouse model using sporozoites of *P. berghei*. The procedures have been previously described.[1–4] Briefly, each compound was ground with a mortar and pestle and suspended in hydroxyethylcellulose and Tween 80 for compounds to be administered PO and those given SC were suspended in peanut oil. Each compound was prepared at different dose levels. Compounds were administered either PO or SC to mice, once a day for three consecutive days, on the day before, 4 h before, and the day after being inoculated with sporozoites of *P. berghei* intravenously. Whole body weights were taken on Day 0 and Day 6 then approximately twice a week for 31 days. A blood film was taken on Day 5 and then approximately twice a week for 31 days. Mice losing greater than about 20% of their body weight were sacrificed. A compound was considered active if only low levels of parasites were found (less than about 10%) in blood films taken on Day 5 or any biweekly for 31 days. Mice alive on Day 31 with no parasites found in any blood films were considered protected. Table 1 summarizes the test results of the new compounds.

4.4.4. Assessment of causal prophylactic/radical curative activities in Rhesus monkeys

The causal prophylactic antimalarial activity of the new derivatives **8e** and **9c** were assessed in a *P. cynomolgi* sporozoites challenged Rhesus monkey model. Detailed procedure of sporozoites harvest and drug tests are described in the previous reports.[1–4] The results are shown in Table 2. Assessment of radical curative activity of the test compounds were carried out using the monkeys developed parasitemia during the causal prophylactic experiments when the test compounds showed no or weak activity. Monkeys were treated with chloroquine (10 mg/kg/day) by oral for seven consecutive days and the test compounds by im for three consecutive days after the parasitemia level reached 5000 parasites/mm.[3] Chloroquine at 10 mg/kg/day × 7 eliminates the blood stage parasites, but not the liver stage hypnozoites. Compounds with antihypnozoite activity will delay the relapse or radically cure the infection. To evaluate the radical curative properties, daily blood samples are followed for 21 days, three times per week for 4 weeks, and then two times weekly until 100 days after the last day of test compound administration. Parasite clearance should occur in all animals treated with chloroquine. Relapse is expected in the control group. Relapse in the treated group indicates failure of the test compounds. Monkeys showed no relapse after 100 days are considered radically cured. Relapses of the control monkeys are treated with chloroquine once daily for 7-days and observe for the second relapse. Relapse in experimental animals and the second relapse of the control monkeys are treated with the standard 7-day oral CQ and primaquine (1.78 mg base/kg). After standard treatment, blood smears were monitored daily for four consecutive days and two times weekly for 2 weeks. The results were shown in Table 3.

Acknowledgments

Material has been reviewed by the Walter Reed Army Institute of Research. There is no objection to its presentation and/or publications. The opinions or assertions contained herein are the private views of the authors, and are not to be construed as official, or as reflecting true views of the Department of the Army or the Department of Defense. This research is supported in part by funding from Military Infections Diseases Research Program (A40191_09_WR), US Army Medical Research and Material Command, Department of Defense, USA., Peer Reviewed Medical Research Program (PRMRP) (Grant # PR054609), and Medicines for Malaria Venture (MMV 04/0013), Geneva, Switzerland. ChemMatCARS Sector 15 is principally supported by the National Science Foundation/Department of Energy under grant number CHE-0535644. Use of the Advanced Photon Source was supported by the US Department of Energy, Office of Science, Office of Basic Energy Sciences, under Contract No. DE-AC02-06CH11357.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.12.028.

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