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Structure identification and prophylactic antimalarial efficacy of 2-guanidinoimidazolidinedione derivatives

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Abstract—The reported synthetic procedure of WR182393, a 2-guanidinoimidazolidinedione derivative with high prophylactic antimalarial activity, was found to be a mixture of three closely related products. Poor solubility of WR182393 in both water and organic solvents and its impractical synthetic method have made the purification and structure identification of the reaction mixture a highly challenging task. The problems were circumvented by prodrug approach involving carbamate formation of the mixture, which enhances the solubility of the mixture in common organic solvents and facilitates the separation and structure determination of the two products. The structures of the two components were determined by X-ray crystallography and NMR of their corresponding carbamates **3a** and **4a**. Additional alkyl carbamates were prepared according to the same approach and two new carbamates **3b** and **4d** were found to possess higher intramuscular (im) efficacy than the parent compound WR182393 against *Plasmodium cynomolgi* in Rhesus monkey.

1. Introduction

Chlorproguanil (**1**) is highly active against primary exoerythrocytic forms of *Plasmodium falciparum* and *P. vivax*. WR182393, a cyclic dicarboxamide derivative of **1** was found to possess prophylactic activity in the mouse Rane assay comparable to that of tafenoquine via subcutaneous administration, where WR182393 demonstrated radical cure (complete elimination of malaria parasites from the body so that relapses cannot occur) at 10 mg/kg.[1,2] It also showed radical cure at 160 mg/kg when dosed orally. In Rhesus monkeys, WR182393 demonstrated both radical curative and causal prophylactic (complete prevention of erythrocytic infection by the administration of drugs that destroy either the sporozoites or the tissue forms of the malaria parasite) activity against *P. cynomolgi* via intramuscular administration (im).[3]

WR182393 was synthesized first by Werbel et al. in 1972 and later by Starks Co.[4] The procedure used to synthesize WR182393 involved treatment of chlorproguanil (**1**) with diethyl oxalate (**2**). The structure of the reaction product was assumed by the manufacture to be compound **3** (Scheme 1). However, this reaction could possibly give a mixture of six structurally closely related products as shown in Scheme 1. There was no convincing analytical data to support the structural assignments. In this study, it was found that WR182393 prepared by Starks Co. comprised at least three compounds as indicated by HPLC and NMR, the starting material **1** plus two compounds out of the six proposed products **3**–**8**. Thus, the biological data derived from WR182393 is the result of a mixture, not a single pure compound. Nevertheless, the active components of WR182393 are potentially valuable leads for medicinal chemists to search for new malaria prophylactic drugs.

Due to the poor solubility of WR182393 in both water and organic solvents, the purification and structure identification of the reaction mixture have been a frustrating task to medicinal chemists. Because prodrug

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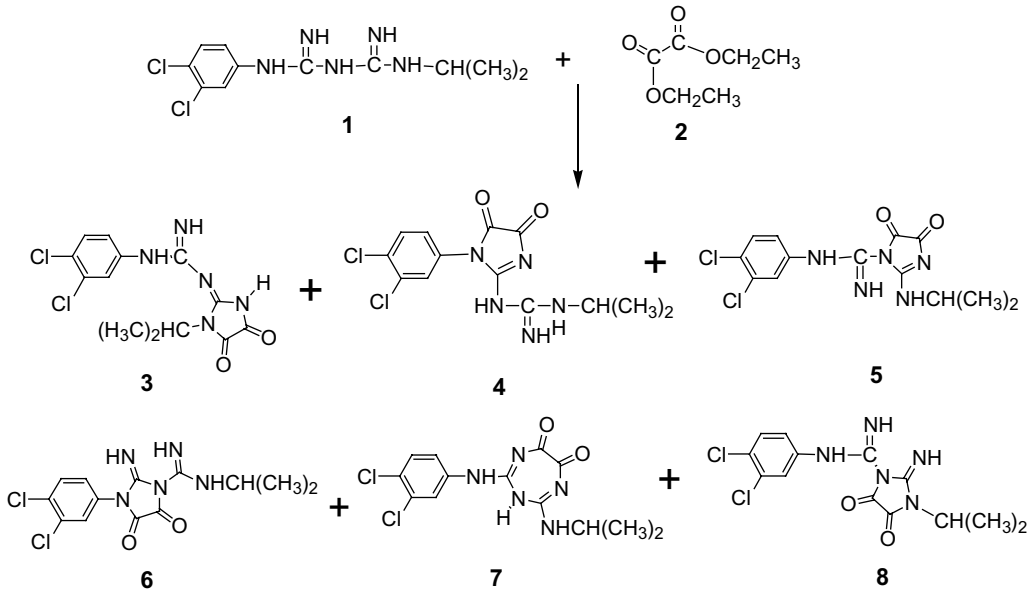
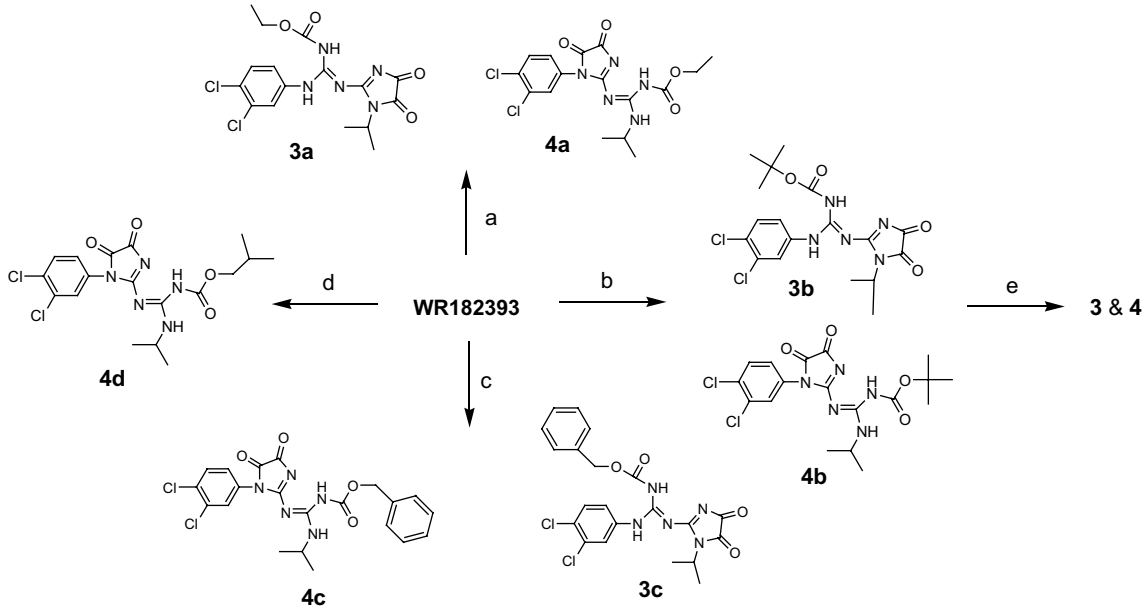


Scheme 1.

strategy is usually adapted to improve drug's solubility, and hence bioavailability, we have successfully utilized this strategy to increase the solubility of WR182393 by carbamate formation, which facilitates the separation, purification, and structure determination of its components.

2. Results and discussion

Initially, WR182393 was treated with ethyl chloroformate in the presence of triethylamine (Scheme 2). The ethylcarbamate obtained was a combination of two products with an estimated ratio of 2:1 as indicated by the NMR spectrum. The resulting two carbamates mixture had much better solubility than WR182393, but had identical R_f values on TLC under various solvent systems and thus, could not be separated by chromatography. However, the differences in their solubility in ethyl acetate facilitated the fractional crystallization to yield, first, the white crystal component **4a**, followed by yellow crystal component **3a**. Since the structures of the two carbamate products are closely related to four other possible products, unambiguous structure identification based solely on NMR data of the carbamate products is very difficult.



Scheme 2. Reagents: (a) ClOCOEt, Et_3N, $CHCl_3$; (b) di-*t*-butyl-dicarbonate, DMAP, $CHCl_3$; (c) dibenzyldicarbonate, DMAP, DMF; (d) $ClOCOCH_2CH(CH_3)_2$, Et_3N, $CHCl_3$; (e) 3 N HCl/EtOAc.

The structures of the two carbamates **3a** and **4a** were, therefore, determined by X-ray crystallography as depicted in Figures 1 and 2. In the ethyl carbamate **3a**, the imidazolidinedione and 3,4-dichlorophenyl rings are separated by an N–C–N chain with the isopropyl group attached to the imidazolidinedione ring. Whereas, in the ethyl carbamate **4a**, the imidazolidinedione and 3,4-dichlorophenyl rings are bonded directly to each other with the isopropyl group remaining part of the side chain. However, many features of their crystalline structures are similar. Both **3a** and **4a** possess large nearly planar portions with the 3,4-dichlorophenyl ring angled around 52° to the planar portion. Besides the 3,4-dichlorophenyl ring, the only nonhydrogen atoms that are not essentially co-planar are the terminal carbon atoms of the isopropyl groups. Both **3a** and **4a** have two intramolecular hydrogen bonds between hydrogen atoms residing on acyclic nitrogen atoms and the imidazolidinedione ring nitrogen and the carbamate carbonyl oxygen atom. Neither structure contained any intermolecular hydrogen bonds.

The structures of **3a** and **4a** were further confirmed by NMR as shown in Figure 3. The chemical shifts of methlyene and methyl protons of the isopropyl group from **3a** and **4a** are distinctively different, with the former (4.45 ppm for –CH– and 1.40 ppm for $–CH_3$) being more down shift than the latter (4.16 ppm for –CH– and 1.25 ppm for $–CH_3$). Molecular model of **4a** indicated that the imidazolidinedione and the 3,4-dichlorophenyl rings are not in the same plane, but rather perpendicular to each other because of the steric hindrance of the bulky guanidino substituents and the isopropyl group is sitting above the 3,4-dichlorophenyl ring. Therefore, all seven isopropyl protons in **4a** are shielded by the phenyl ring electrons, resulting in the observed up field chemical shifts. Whereas both 3,4-dichlorophenyl and the imidazolidinedione rings of **3a** are separated by a guanidine side chain, no electronic interactions between the isopropyl group and the aromatic ring are expected. Other distinct difference in chemical shifts between **3a** and **4a** was that the aromatic proton Ha of **4a** (7.56 ppm) is 0.3 ppm up field shifted relative to the corresponding proton of **3a** (7.84 ppm), possibly due to shielding effects of the carbonyl oxygen of the imidazolidinedione ring in **4a**. NMR data is clearly in agreement with the structure assignment of **3a** and **4a** by X-ray crystallography.

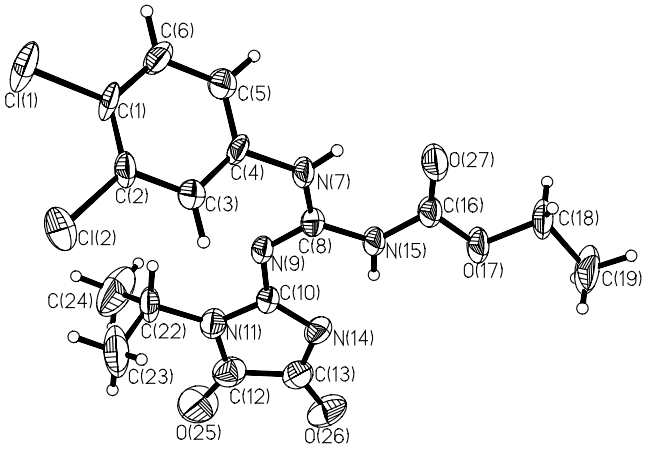


Figure 1. Thermal ellipsoid plot of crystalline **3a**, *N*-(3,4-dichlorophenyl)-*N′*-ethylcarbonyl-*N″*-(1-isopropyl-4,5-dioxo-imidazolidin-2-ylidene)-guanidine, showing the atom numbering scheme used for the X-ray crystallographic data. Atoms N7, C8, N9, C10, N11, C12, C13, N14, N15, C16, O17, O25, O26, and O27 are essentially co-planar with atom N7 showing the largest deviation (0.13 Å) off a least-squares plane through these atoms. The phenyl ring is angled such that the C3–C4–N7–C8 torsion angle is 52.8°. The crystal structure contained only intramolecular hydrogen bonds, between N7–H7 and O27 and N15–H15 and N14. The N7–H7···O27 angle is 131.1°, H7···O27 distance is 2.029 Å, and N7···O27 distance is 2.674 Å. The N15–H15···N14 angle is 139.8°, H15···N14 distance is 1.879 Å, and N15···N14 distance is 2.660 Å.

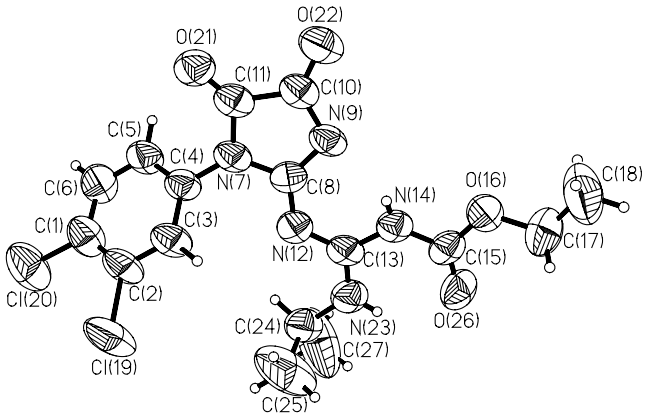


Figure 2. Thermal ellipsoid plot of crystalline **4a**, *N*-[1-(3,4-dichlorophenyl)-4,5-dioxo-imidazolidin-2-ylidene]-*N′*-isopropyl-*N″*-(ethylcarbonyl)guanidine, showing the atom numbering scheme used for the X-ray crystallographic data. Atoms N7, C8, N9, C10, C11, N12, C13, N14, C15, O16, O21, O22, N23, and O26 are essentially co-planar with atoms O16 and O22 showing the largest deviations (0.14 Å) off a least-squares plane through these atoms. The phenyl ring is angled such that the C3–C4–N7–C8 torsion angle is 50.7°. The crystal structure contained only intramolecular hydrogen bonds, between N14–H14 and N7 and N23–H23 and O26. The N14–H14···N9 angle is 148.7°, H14···N9 distance is 1.966 Å, and N14···N9 distance is 2.653 Å. The N23–H23···O26 angle is 129.8°, the H23···O26 distance is 2.077 Å, and N23···O26 distance is 2.679 Å. The large thermal ellipsoids for the terminal methyl groups indicate that these methyl groups are not tightly sterically restricted in the crystalline packing.

Several new carbamates (**3b**, **4b–d**) were prepared and separated by the same approach. The differences in chemical shifts between **3a** and **4a** were, likewise, observed in the new carbamates **3b**,**c** and **4b–d**. The *t*-Boc groups of the *t*-Boc carbamates **3b** and **4b** were hydrolyzed in 3 N HCl/ethyl acetate[5] resulting in regeneration of the parent components **3** and **4**, respectively. The structures of **3** and **4** were further confirmed by NMR, MS, and elemental analysis.

The causal prophylactic antimalarial activity of two carbamates **3b** and **4d** were assessed against *P. cynomolgi* in Rhesus monkey according to the methodology described by Schmidt for the evaluation of causal prophylaxis and radical cure.[6–8] Two monkeys were used in each test group. The control animals developed parasitemia in

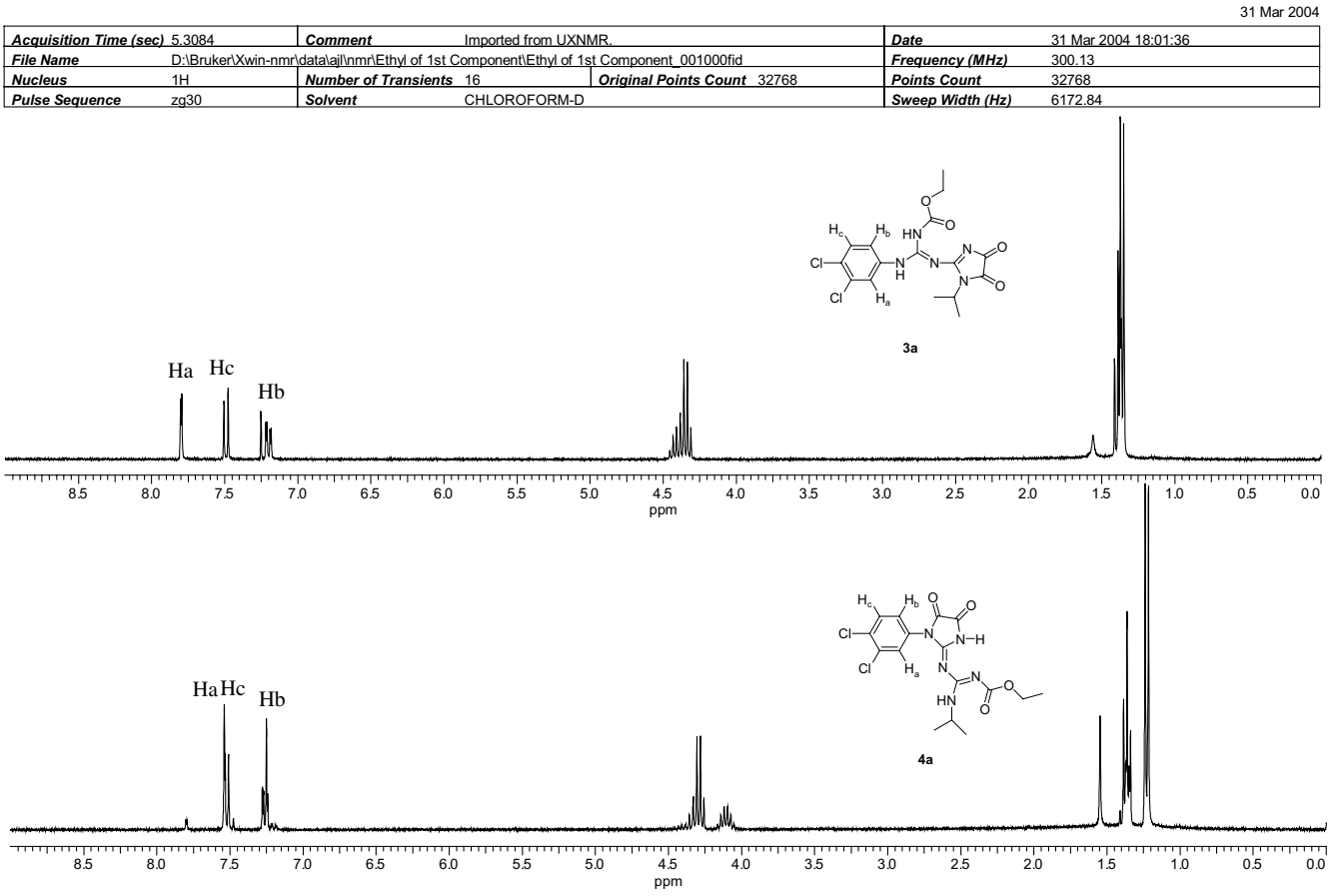


Figure 3. NMR spectrum of **3a** and **4a**.

about 10 days after inoculation of about 1×10^6 *P. cynomolgi* sporozoites harvested from *Anopheles dirus*. All the monkeys received three treatments, once a day for three consecutive days beginning a day before $(-1, 0, +1)$ the sporozoites challenge. The control monkeys (group 1) received HECT and the experimental animals received testing compound by using 8 Fr., 15-in. long nasogastric tubes. The results are shown in Table 1. The positive control drug, WR182393, at 30 mg/kg given via intramuscular (im) injection, protected one monkey and delayed parasitemia formation in the other monkey for 75 days, which confirmed a previous report that this compound possessed im causal prophylactic and radical curative activity against *P. cynomologi*. Both monkeys receiving 30 mg/kg by im of compound **4d** were protected and stayed parasite free 100 days after treatment. At lower dose of 10 mg/kg, one monkey remained parasite free after day 100, but the other monkey developed parasitemia after day 47. Compound **3b** showed the best prophylactic efficacy among the three com-

Table 1. Causal prophylactic activities of **3b** and **4d**

Monkey no.	Group	Drug	Day of parasitemia	Results[a]
DA889	Control	None (DMSO)	Parasitemia on day 10	Valid control
DA891			Parasitemia on day 13	Valid control
DA888	1	WR182393, 30 mg/kg	Parasitemia on day 75	Delay the parasitemia
DA895			Parasite free on day 100	Causal prophylaxis
DA874	2	**4d**, 30 mg/kg	Parasite free on day 100	Causal prophylaxis
DA894				
DA880	3	**4d**, 10 mg/kg	Parasitemia on day 47	Delay the parasitemia
DA883			Parasite free on day 100	Causal prophylaxis
DA873	4	**3b**, 30 mg/kg	Parasite free on day 100	Causal prophylaxis
DA877				
DA875	5	**3b**, 10 mg/kg		
DA878				

[a] Treated monkeys remained parasite free for 100 days after the treatment were considered causal prophylaxis or protected.

pounds tested, protecting all four monkeys dosing with either 10 or 30 mg/kg of the compound. Criteria used to define 'cure' in the test is that the treated monkeys remained parasite free for 100 days after the treatment were considered cured or protected.

Preliminary data indicated that none of the three compounds showed significant oral protective activity up to 60 mg/kg in Rhesus monkey model. However, good oral activity was observed in mouse model. The mouse oral efficacy data will be published later in a separate report.

3. Conclusion

In summary, we successfully separated, purified, and identified the components of WR182393 through carbamate prodrug approach. Pure carbamates **3b** and **4d** possessed superior prophylactic efficacy to that of WR182393 mixture in tests against *P. cynomolgi* in Rhesus monkey by im. Due to promising prophylactic efficacy, an unambiguous synthesis for **3** and **4** from commercially available chemicals had been accomplished in our laboratory. A series of new carbamates derived from pure compounds **3** and **4** were prepared and tested in a sporozoites challenged mouse model. The new synthetic methodologies of compound **3** and **4** and the biological results of new derivatives will be reported separately elsewhere.

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 microns silica gel plates (Analtech, Newark, DE). Visualization of the developed chromatogram was performed by UV absorbance, or spreading with aqueous potassium permanganate, or ethanolic anisaldehyde. Liquid chromatography was performed using a Horizon HPFC System (Biotage, Charlottesville, VA) with Flash 25 or 40 M cartridges (KP-Sil™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 were recorded in deuteriochloroform, unless otherwise noted, on a Bruker Avance 300 and Bruker Avance 600 spectrometer (Bruker Instruments, Inc., Wilmington, DE). Chemical shifts are reported in parts per million on the δ scale from an internal standard of tetramethylsilane. 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.

4.1. Procedure for preparation of ethyl carbamates

To a suspension of WR182393 (4 g, 11.7 mmol) in 80 mL of $CHCl_3$ was treated with Et_3N (6.2 mL, 4 equiv) and ethyl chloroformate (4.4 mL, 4 equiv). The reaction mixture was stirred at room temperature overnight, washed with water, and the chloroform layer was dried over Na_2SO_4 and concentrated. The residue was applied to a silica gel flash column chromatography and eluted with 2.5% EtOAc/$CHCl_3$. The ethyl carbamate obtained (2.8 g) was a mixture of two compounds with a ratio of approximately 2:1 (A:B) as indicated by NMR. Fractional crystallization of the mixture from ethyl acetate gave first isomer B (850 mg, 18%), followed by isomer A (1.3 g, 27%). The structures of the two products were determined by NMR and X-ray crystallography as **3a** (isomer A) and **4a** (isomer B).

Ethyl carbamate **3a**: mp 181 °C. ^{1}H NMR ($CDCl_3$, 600 Hz): δ 13.20 (s, 1H, –NH), 11.17 (s, 1H, –NH), 7.84 (d, J = 2.4 Hz, 1H), 7.53 (d, J = 8.6 Hz, 1H), 7.24 (dd, J = 8.6, 2.4 Hz, 1H), 4.45 (m, 1H), 4.38 (q, J = 7.1 Hz, 2H), 1.43 (t, J = 7.1 Hz, 3H), 1.40 (d, J = 7.0 Hz, 6H). Anal. ($C_{16}H_{17}N_5O_4Cl_2$) C, H, N.

Ethyl carbamate **4a**: mp 257 °C. ^{1}H NMR ($CDCl_3$, 600 Hz): δ 7.56 (d, J = 2.4 Hz, 1H), 7.56 (d, J = 8.6 Hz, 1H), 7.29 (dd, J = 2.4 and 8.6 Hz, 1H), 4.32 (q, J = 7.1 Hz, 2H), 4.16 (m, 1H), 1.39 (t, J = 7.1 Hz, 3H), 1.25 (d, J = 6.6 Hz, 6H). Anal. ($C_{16}H_{17}N_5O_4Cl_2$) C, H, N.

Benzyl carbamates **3c** and **4c** and isobutyl carbamate **4d** were prepared using the same procedure.

Benzyl carbamate **3c**: 25%, mp 235 °C. ^{1}H NMR ($CDCl_3$, 600 Hz): δ 7.81 (s, 1H), 7.52 (d, J = 8.70 Hz, 1H), 7.44 (m, 5H), 7.23 (d, J = 8.70 Hz, 1H), 5.32 (s, 2H), 4.42 (m, 1H), 1.39 (d, J = 7.0 Hz, 6H). Anal. ($C_{21}H_{19}N_5O_4Cl_2$) C, H, N.

Benzyl carbamate **4c**: 16%, mp 229 °C. ^{1}H NMR (*d*-DMSO, 600 Hz): δ 7.79 (d, J = 8.61 Hz, 1H), 7.75 (d, J = 2.30 Hz, 1H), 7,48–7.34 (m, 6H), 5.30 (s, 2H), 3.97 (m, 1H), 1.17 (d, J = 6.5 Hz, 6H). Anal. ($C_{21}H_{19}N_5O_4Cl_2$) C, H, N.

Isobutyl carbamate **4d**: 31%, mp 260 °C. ^{1}H NMR (600 MHz, $CDCl_3$): δ 12.44 (s, 1H, –NH), 9.24 (d, J = 7.2 Hz, 1H, –NH), 7.55 (d, J = 2.4 Hz, 1H), 7.54 (d, J = 8.6 Hz, 1H), 7.28 (dd, J = 8.6 and 2.4 Hz, 1H), 4.12 (m, 1H), 4.02 (d, J = 6.8 Hz, 2H), 2.05 (m, 1H), 1.24 (d, J = 6.6 Hz, 6H), 0.99 (d, J = 6.7 Hz, 6H). Anal. ($C_{18}H_{21}Cl_2N_5O_4$) C, H, N, Cl.

4.2. Procedure for preparation of *t*-butyl carbamates

To a suspension of WR182393 (500 mg, 1.46 mmol) in DMF (10 mL) was added DMAP (18 mg, 0.2 equiv) and di-*tert*-butyl-dicarbonate (1.27 g, 4 equiv). The reaction mixture was stirred at room temperature overnight and partitioned in $CHCl_3/H_2O$. The organic layer was successively washed with brine and water, dried over Na_2SO_4, filtered, and concentrated. The crude products were purified by silica gel flash column chromatography (2.5% EtOAc/$CHCl_3$) and crystallized from EtOAc to give pure isomer A (**3b**) (54 mg, 26%) and isomer B (**4b**) in about equal yield.

t-Boc carbamate **3b**: mp 223 °C. ^{1}H NMR ($CDCl_3$, 600 Hz): δ 7.84 (d, J = 2.3 Hz, 1H), 7.52 (d, J = 8.6 Hz, 1H), 7.25 (dd, J = 8.6, 2.3 Hz, 1H), 4.44 (m, 1H), 1.60 (s, 9H), 1.39 (d, J = 7.0 Hz, 6H). Anal. ($C_{18}H_{21}N_5O_4Cl_2$) C, H, N.

t-Boc carbamate **4b**: mp 227 °C. ^{1}H NMR ($CDCl_3$, 300 Hz): δ 7.55 (d, J = 2.3 Hz, 1H), 7.54 (d, J = 7.50 Hz, 1H), 7.27 (dd, J = 2.3, 7.50 Hz, 1H), 4.15 (m, 1H), 1.53 (s, 9H), 1.22 (d, J = 6.6 Hz, 6H). Anal. ($C_{18}H_{21}N_5O_4Cl_2$) C, H, N.

4.3. Hydrolysis of carbamate

t-Boc carbamate (**3b**) (196 mg, 0.44 mmol) was dissolved in 3 N HCl/EtOAc (3 mL). After 2 h, the mixture was neutralized with satd $NaHCO_3$ and filtered. The yellow solid was washed successively with water and chloroform to yield pure **3** (140 mg, 93%).

Compound **3**: mp 227 °C. ^{1}H NMR (DMSO-d_6, 600 Hz) δ 7.78 (s, 1H), 7.69 (d, J = 8.4 Hz, 1H), 7.31 (d, J = 8.4 Hz, 1H), 4.39 (s, 1H), 1.33 (d, J = 6.7 Hz, 6H). Anal. ($C_{13}H_{13}N_5O_2Cl_2 \cdot 1/4CH_3CO_2C_2H_5$) C, H, N.

Compound **4** was also prepared by the hydrolysis of **4b** under the same conditions as that of compound **3**. Yield 89%, mp 244 °C. ^{1}H NMR (DMSO-d_6, 600 Hz) δ 8.72–8.67 (m, 1H), 7.76 (d, J = 8.5 Hz, 1H), 7.72 (d, J = 2.0 Hz, 1H), 7.42 (dd, J = 8.5, 2.0 Hz, 1H), 3.76 (m, 1H), 1.14 (d, J = 6.1 Hz, 6H). Anal. ($C_{13}H_{13}N_5O_2Cl_2$) C, H, N.

4.4. X-ray crystal structure determination

The X-ray samples were colorless plates (**3a**: $0.7 \times 0.35 \times 0.12$ mm; **4a**: $0.45 \times 0.42 \times 0.06$ mm) crystallized from methanol. Data collection was performed at room temperature (293 ± 2 K) on a Bruker P4 diffractometer using Cu Kα radiation and a graphite monochrometer in the incident beam. Reflections used to refine the unit cell parameters by least-squares methods are as follows: **3a**, 31 reflections in the range of $12° \leqslant \theta \leqslant 40°$; **4a**, 27 reflections in the range of $14° \leqslant \theta \leqslant 26°$. Crystal data **3a**: $C_{16}H_{17}C_{12}N_5O_4$, FW = 414.25, monoclinic, $P2(1)/n$, a = 14.919(2) Å, b = 7.613(1) Å, c = 17.504(3) Å, β = 102.79(2)°, V = 1938.7(5) Å^3, Z = 4. Crystal data **4a**: $C_{16}H_{17}C_{12}N_5O_4$, FW = 414.25, triclinic, P-1, a = 7.924(2) Å, b = 9.704(2) Å, c = 13.375(2) Å, α = 102.90(1)°, β = 96.18(1)°, γ = 97.39(2)°, V = 984.2(4) Å^3, Z = 4. The data were collected using the ω scan technique with a variable scan rate ranging from 3°/min minimum to 60°/min maximum depending upon the intensity of the reflection. Three reflections were checked as intensity controls every 97 reflections and remained constant within 3.2% for **3a**, 3.5% for **4a**. No absorption correction was applied. The structures were solved using direct methods.[9,10] Full matrix least-squares refinement[11] was performed on coordinates and anisotropic thermal parameters for the nonhydrogen atoms, isotropic thermal parameters for the hydrogen atoms using reflections for which $|F_O| > 4\sigma(F_O)$. Hydrogen atoms H7 and H15 for **3a** and H14 and H23 for **4a** were located in the difference maps. The remaining hydrogen atoms were placed in idealized positions, and during refinement, the coordinates of these hydrogen atoms rode with the coordinates of the carbon to which they are attached. Final bond distances and angles were all within expected and acceptable limits.

Acknowledgements

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 author, and are not to be construed as official, or as reflecting true views of the Department of the Army or the Department of Defence.

References and notes

1. Curd, F. S. H.; Davey, D. G.; Rose, F. L. *Ann. Trop. Med. Parasitol.* **1945**, *39*, 208.
2. Shanks, G. D.; Oloo, A. J.; Aleman, G. M.; Ohrt, C.; Klotz, F. W.; Braitman, D.; Horton, J.; Brueckner, R. *Clin. Infect. Dis.* **2001**, *33*, 1968.
3. Corcoran, K. D.; Hansukjariya, P.; Sattabongkot, J.; Ngampochjana, M.; Edstein, M. D.; Smith, C. D.; Shanks, G. D. *Am. J. Trop. Med. Hyg.* **1993**, *49*, 473.
4. Contractors of Walter Reed Army Institute of Research, U.S. Army.
5. Stahl, G. L.; Walter, R.; Smith, C. W. *J. Org. Chem.* **1978**, *43*, 2285.
6. Schmidt, L. H.; Fradkin, R.; Genther, C. S.; Rossan, R. N.; Squires, W. *Am. J. Trop. Med. Hyg.* **1982**, *31*, 612.
7. Schmidt, L. H.; Fradkin, R.; Genther, C. S.; Rossan, R. N.; Squires, W., II. *Am. J. Trop. Med. Hyg.* **1982**, *31*, 646.
8. Schmidt, L. H.; Fradkin, R.; Genther, C. S.; Hughes, H. B., III. *Am. J. Trop. Med. Hyg.* **1982**, *31*, 666.
9. Karle, J.; Karle, I. L. *Acta Crystallogr.* **1966**, *21*, 849–859.
10. Sheldrick, G. M. Crystallographic Algorithms for Mini and Maxi Computers. In *Crystallographic Computing*; Sheldrick, G. M., Krüger, C., Goddard, R., Eds.; Oxford University Press: Oxford, 1985; Vol. 3, pp 175–179, Sheldrick, G. M. *SHELXTL software*; University of Göttingen, Federal Republic of Germany, 1990.
11. Sheldrick, G. M. *SHELX-97*; University of Göttingen, Federal Republic of Germany, 1997.