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Synthesis and Antimalarial Activity of 2-Guanidino-4-oxoimidazoline Derivatives

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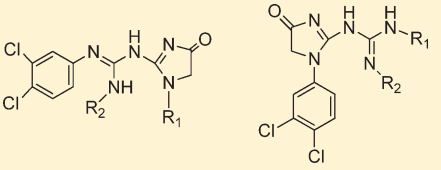
Supporting Information

ABSTRACT: A series of 2-guanidino-4-oxoimidazoline (deoxo-IZ) derivatives was prepared and showed potent antimalarial activities in rodent and Rhesus models. Compound **8e**, the most potent analogues of this series, is the first non-8-aminoquinoline antimalarial that demonstrated radical curative activity in non-human primate by oral route and showed causal prophylactic activity comparable to that of the commonly used clinical drugs in Rhesus monkeys infected with sporozoites of *Plasmodium cynomolgi*. The metabolic stability and metabolites profile indicated that the new deoxo-IZ derivatives (**8**) may act as prodrugs of the corresponding IZ (**1** and **2**) derivatives.



R_1 = -CH_3, -$CH(CH_3)_2$, or -$C(CH_3)_3$
R_2 = alkyl or -CORa
Ra = alkyl or alkoxy

INTRODUCTION

Multiple drug resistance and lack of safe drugs to prevent or to radically cure malaria diseases continue to pose challenges to the containment of this deadly disease that has threatened the life of millions of people in the underdeveloped world.[1−5] Widespread drug resistance to chloroquine, the first line antimalarial drug, was reported in Southeast Asia and South America.[6−10] Central nervous system (CNS) toxicity of mefloquine[11] and hemolytic side effects of primaquine (PQ) and tafenoquine (TQ) in glucose 6-phosphate dehydrogenase (G6PD) deficiency patients[7,8] have compromised the clinical value of these otherwise highly effective malaria therapeutic drugs. Multiple drug resistance in *Plasmodium falciparum* malaria continues to pose special problems for targeting the blood stages of malaria. Our malaria drug research teams at the Walter Reed Army Institute of Research are placing emphasis on developing new chemical entities with true causal prophylactic and/or radical curative properties, stopping malaria before blood stages emerge and cause clinical disease. With the exception of quinoline esters,[12] only the 8-aminoquinoline drugs such as PQ or TQ[13−15] have activity against the liver stages of *Plasmodium vivax* and *P. falciparum* malarias. However, the 8-aminoquinoline drugs can cause serious lethal hemolytic side effects in G6PD deficient patients.[7,8,13] Therefore, there is an eminent need for new and safe antimalarial drugs to combat the parasites and protect the tourists traveling in the endemic areas of the world.

Recently, a series of new 2-guanidinoimidazolidinedione (IZ) derivatives (**1** and **2**, Figure 1) were demonstrated in our laboratory to possess causal prophylactic antimalarials activity in Rhesus monkeys infected with *P. cynomolgi* sporozoites.[16−20] Carbamates **3**, the most active compounds of this class, protect monkeys infected with *P. cynomolgi* sporozoites at a dose of 10 mg/kg × 3 days by im dosing. Nevertheless, the IZ derivatives showed very weak or no in vitro cell growth inhibition against blood stage malaria *P. falciparum* and are inactive in Thompson mouse test against *P. berghei*, a blood stage rodent malaria.[17,18] To the best of our knowledge, this is the first class of antimalarial agents possessing activity against the liver stage malaria exclusively. From the drug resistant development point of view, drugs used exclusively for prophylaxis have much less chance of exposure to parasites than those used for treatment and thus have fewer problems of developing drug resistance. However, carbamate **3** showed poor activities by oral administration; it protects the treated monkeys for only 3 days vs untreated control. The acid sensitive carbamate group of **3** and **4** was considered the cause of poor oral activity. To overcome the acid stability problem, chemically more stable carboxamide analogues **5** and **6** were prepared and again found to be active only by intramuscular injection (im), not by oral (po) dosing.[20] Pharmacokinetic studies of carboxamides **5** and **6** in rats revealed that the former is much more stable metabolically than the latter with $t_{1/2}$ of 2−3 h and <20 min, respectively, and both were converted to the same *s*-triazine derivatives.[20] This transformation was also observed when both compounds were incubated in microsomal preparations and in phosphate buffer solution, indicating that conversion of carboxamides **5** and **6** to *s*-triazine **7** is a chemical rather than an enzymatic reaction.[20] The facile transformation of IZ molecules **5** and **6** to *s*-triazine **7** revealed that the five-membered imidazolinedione ring, though an amide, is rather labile chemically, especially, when the five-membered imidazolinedione ring carries electron withdrawing substituents, such as 3,4-dichlorophenyl and carboxamide, as in **5** and **6**. The imidazolinedione rings of both **5** and **6** are readily hydrolyzed to biguanides which cyclized to form *s*-triazine derivatives.[20] In this study, we report the synthesis and antimalarial activity of a series

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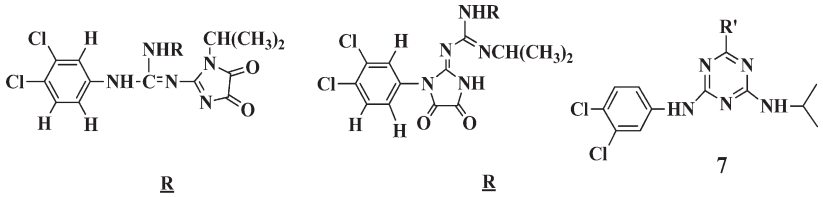


Figure 1. Chemical structures of imidazolinediones and *s*-triazine derivatives.

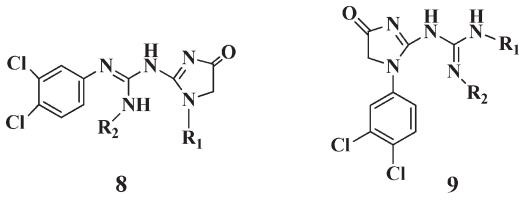


Figure 2. New 4-oxoimidazoline derivatives.

of chemically more stable 2-guanidino-4-oxoimidazoline derivatives. The unstable imidazolinedione rings of the IZ molecules (**1**−**6**) were replaced with a chemically more stable 4-oxoimidazoline ring (**8**, **9**), as shown in Figure 2.

CHEMISTRY

The new compounds **8** and **9** are deoxo analogues of the imidazolinedione derivatives (IZ) which exhibited potent causal prophylactic activity and curative activity in Rhesus models by intramuscular injection. Though oral activity was observed in mice at high dose (>160 mg/kg × 3), IZ compounds **1**−**6** showed no oral activity in the Rhesus model. The lack of oral activity was at least in part due to the short plasma half-life which in turn is due to the chemical instability of the five-membered imidazolinedione ring of the IZ compounds. Since the 4-oxoimidazolines (deoxo-IZs) are chemically more stable than 4,5-imidazolinediones (IZs), deoxo-IZ analogues **8** and **9** may possess better oral activity than the corresponding IZ derivatives (**3**−**6**). Deoxo-IZ (**8a**−**c**), *N*-carbamates (**8d**,**g**), and *N*-carboxamides (**8h**,**i**) were prepared according to Scheme 1. The starting materials *N*-alkylaminoacetic acid (**10a**−**c**) and 1-alkyl-2-thiohydantoin (**11a**−**c**) were prepared according to the reported procedures.[21,22] The starting materials **10a**−**c** were obtained in over 90% yield by treatment of hydroxyacetonitrile with primary amine at room temperature for 1 h, followed by NaOH hydrolysis. Fusion of *N*-alkylaminoacetic acids **10a**,**b** with NH_4SCN at 140 °C gave compounds **11a**,**b** in 65% yield. Although treatment of chloroacetic acid with *tert*-butylamine gave a decent yield of *N*-*tert*-butylaminoacetic acid (**10c**), fusion of **10c** with NH_4SCN gave poor yields of compound **11c**. Regioselective S-alkylation of **11a**−**c** with 1.5 equiv of CH_3I gave **12a**−**c** HI salt in good yield.[23] The salts **12a**−**c** were converted to free base compounds **13a**−**c** in 95% yield by basification with K_2CO_3. The desired guanidine derivatives (**8a**−**c**) were readily prepared in good yield by coupling of 1-alkyl-2-methylsulfanylimidazolin-4-one (**13a**−**c**) with 3,4-dichlorophenylguanidine.

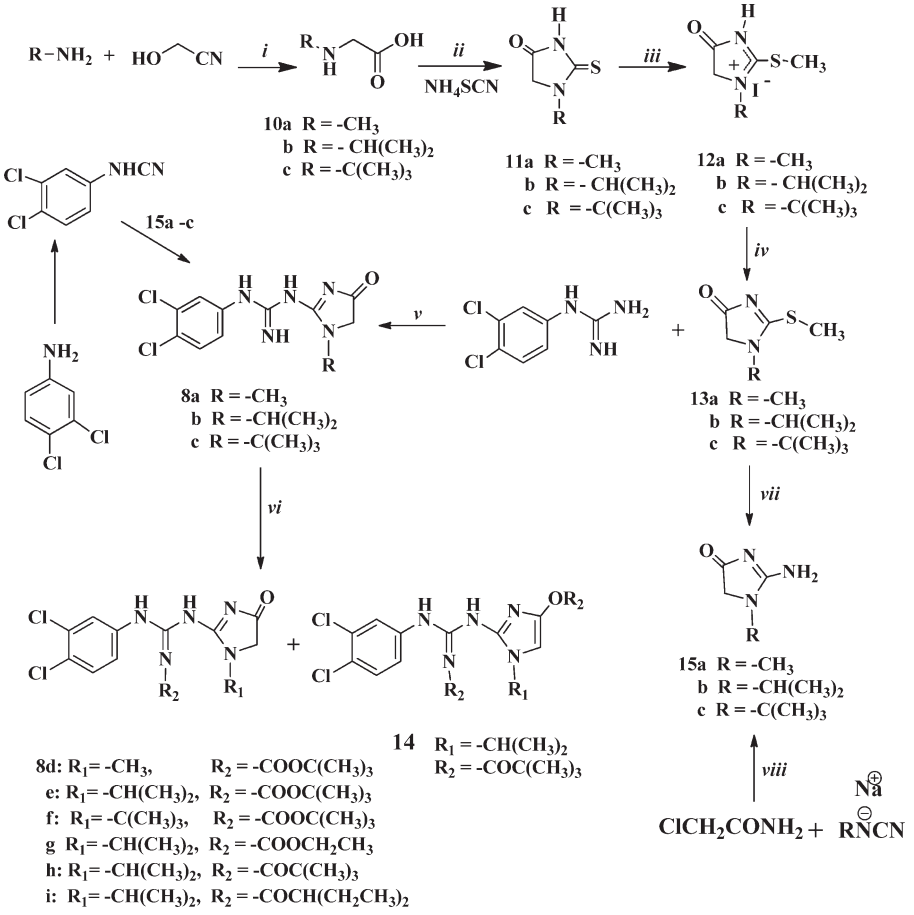
Carbamates **8d**−**g** was prepared by treatment of **8a**, **8b**, or **8c** with 1 equiv of di-*tert*-butyl dicarbonate or alkyl chloroformate under basic conditions. Similarly, treatment of compound **8b** with 1 equiv of trimethylacetyl chloride or 2-ethylbutyryl chloride resulted in the formation of carboxamide **8h** or **8i**, respectively. However, it is interesting to note that with treatment of **8b** with more than 4 equiv of acyl chloride, carboxylation of the 4-oxo and the guanidine amino group took place to form compound **14**. For example, treatment of **8b** with 4 equiv of trimethylacetyl chloride at room temperature for 2 h in the presence of Et_3N and DMAP gave **14** in 95% yield. The chemical structure of the product **14** was determined by ^1H NMR, ^{13}C NMR, and LC/MS. In the ^1H NMR spectra of **8h**, nine signals were observed as follows: two NH protons at 14.01 and 11.59 ppm (N-H), three aromatic protons at 8.10, 7.40, and 7.24 ppm -(aromatic protons), and aliphatic protons at 4.54 (-CH-), 3.81 (CO-CH_2-), 1.36 (3 × CH_3), and 1.26 ppm (2 × CH_3). In the ^1H NMR spectra of **14**, the methylene (-$COCH_2$-) proton signals at 3.81 ppm observed in **8h** disappeared; a new olefinic proton signal at 6.65 ppm and a new *tert*-butyl group signal at 1.33 ppm were observed. Likewise, in the ^{13}C NMR spectra of **14**, the methylene carbon signal of **8h** at 44.90 ppm vanished and a new olefinic signal at 97.6 ppm appeared.

2-Amino-1-isopropylimidazolin-4-one (**15b**) was prepared either by treatment of 2-methylsulfanylimidazolin-4-one (**13b**) with ammonia in methanol[23] or by treatment of sodium isopropylcyanamide with chloroacetamide in CH_3CN[24]. The latter method provided the product in one step from the commercially available materials in 80−85% yield, while the former method required multiple-step synthesis from hydroxyacetonitrile. Both methods were used to prepare **15a**−**c** in good yield.

The isopropyl group was considered an essential substituent at the N_1-position of IZ antimalarials **1**−**6** in previous studies. To further examine the importance of the isopropyl group in the deoxo-IZ analogues, a number of *N*-CH_3 and *N*-$C(CH_3)_3$ analogues **8a** and **8c** and their carbamates **8d** and **8f** were designed and prepared as shown in Scheme 1.

2-Amino-1-methylimidazolin-4-one (**15a**) and 2-amino-1-*tert*-butylimidazolin-4-one (**15c**) were prepared using the same method as for the synthesis of 2-amino-1-isopropylimidazolin-4-one (**15b**). Compounds **8a** and **8c** were also prepared in moderate yields (50−60%) by heating **15a** or **15c** with 3,4-dichlorophenylcyanamide in *n*-propanol. Since fusion of **10c** with NH_4SCN gave poor yield of **11c**, compound **8c** was mainly prepared by treatment of **15c** with 3,4-dichlorophenylcyanamide in *n*-propanol. (Scheme 1).

Scheme 1. Synthesis of 2-Guanidino-4-oxoimidazoline Derivatives 8a−i[a]



[a] Reagents and conditions: (i) NaOH/H_2O, 90%; (ii) 140 °C, fusion, 4 h, 70%; (iii) CH_3I, DMF, 10 h, 25 °C, 90%; (iv) K_2CO_3, H_2O; (v) EtOH, 82 °C, 24 h, 75%; (vi) RCOCl or $(RO)_2C{=}O$, Et_3N, DMF, DMAP, 25 °C, 50−65%; (vii) 7.0 M NH_3 in MeOH, 70 °C, 4 h, 85%; (viii) CH_3CN, 70 °C, 4 h, 80%.

While carbamates **8d**−**g** and carboxamides **8h**,**i** of the deoxo-IZs were prepared by derivatization of biguanides **8a**−**c** as shown in Scheme 1, the synthesis of the *N*-alkyl analogues (**8j**−**n**) were prepared by coupling of *S*-methyl-*N*-alkyl-*N*′-(3,4-dichlorophenyl)thioureas **18a**−**d** and **15a**−**c** in one step as shown in Scheme 2. The free base, 2-methylisothioureas **18a**−**d** were prepared via the reaction of 3,4-dichlorophenyl isothiocyanate (**16**) with a variety of primary amines to form thiourea derivatives (**17a**−**d**) followed by methylation with CH_3I.[25] The desired *N*-alkyl guanidine derivatives **8j**−**n** were readily prepared by coupling of the free base (**18a**−**d**) with 2-aminoimidazolines **15a**−**c**. After the samples were heated at 100 °C for 24 h, the substituted guanidine derivatives (**8j**−**n**) were obtained in moderate to good yield (50−75%). It was noted that the coupling reaction required base as catalyst to give good yields. Likewise, preparation of the cyclic amine analogues was achieved by treatment of isothiocyanate with pyrrolidine and piperidine to give the corresponding thiourea derivatives **19a** and **19b**, respectively. Reaction of **19a**,**b** with CH_3I afforded *S*-methylthiourea HI salts **20a**,**b** which were converted to free base prior to coupling with **15b** to afford the desired derivatives **21a**,**b** in moderate yields (41−50%) (Scheme 2).

Our previous structure−activity relationship (SAR) studies indicated that derivatives of **1** are chemically more stable than the corresponding derivatives of **2**. However, the latter compounds are more active than the former in exoerythrocytic mouse tests.[17,18] On the contrary, derivatives of compound **1** are more active than the corresponding less stable derivatives of **2** in the Rhesus monkey test. Since replacement of one carbonyl group in the imidazolidin-4,5-dione derivatives (**1**−**6**) with a methylene group will result in chemically more stable imidazolidin-4-one analogues, a series of deoxo analogues of compound **2** derivatives, **9a**−**e** and **25a**−**c**, were also prepared and tested in this study.

N-[1-(3,4-Dichlorophenyl)-4-oxo-4,5-dihydro-1*H*-imidazol-2-yl]-*N*′-isopropylguanidine (**9a**) and its carbamate (**9b**,**c**) and carboxamide (**9d**,**e**) derivatives were prepared as illustrated in Scheme 3. 3,4-Dichlorophenylcyanamide (**22**) was prepared by a known methods[26] and was converted to sodium salt **23** using sodium metal in absolute ethanol before reacting with chloroacetamide in CH_3CN to give 2-amino-1-(3,4-dichlorophenyl)-1,5-dihydroimidazolin-4-one (**24**). Treatment of compound **24** with isopropylcyanamide in 1-propanol resulted in formation of guanidine derivative **9a** which was converted to carbamates **9b**−**c** and carboxamides **9d**−**e** in moderate yields (40−50%). *N*-Alkyl-*N*′-(3,4-dichlorophenyl)biguanidine analogues **25a**−**c** were prepared by coupling of 2-amino-3-(3,4-dichlorophenyl)imidazolin-4-one (**24**) with compounds **18a**−**d** in the presence of K_2CO_3.

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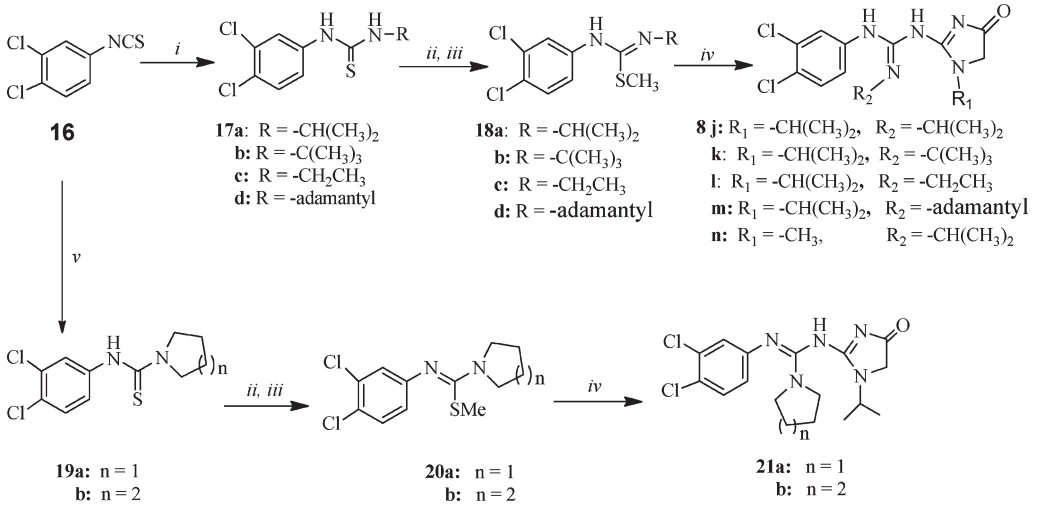
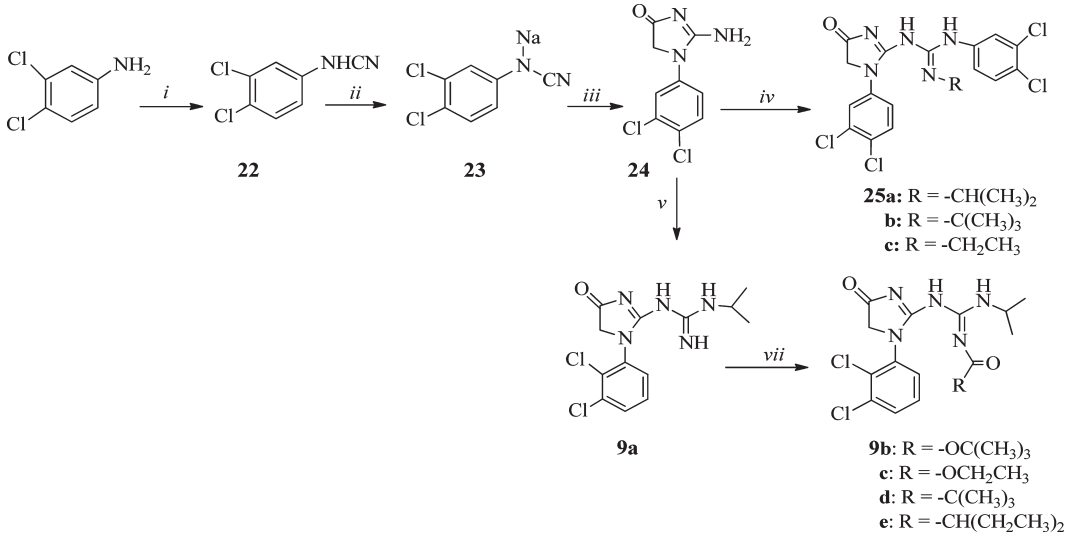
Scheme 2. Synthesis of *N*-(3,4-Dichlorophenyl)-*N′*-subistituted-*N″*-(1-isopropyl-4-oxo-4,5-dihydro-1*H*-imidazol-2-yl)guanidines 8j−n and 21a,b[a]



[a] Reagents and conditions: (i) RNH_2, CH_2Cl_2, 25 °C, 2 h; (ii) CH_3I, acetone, reflux, 2 h; (iii) Na_2CO_3, H_2O, 25 °C; (iv) **15a−c**, K_2CO_3, $CH_3CH_2CH_2OH$, reflux, 24 h; (v) RRNH, CH_2Cl_2, 25 °C, 2 h.

Scheme 3. Synthesis of *N*-[1-(3,4-Dichlorophenyl)-4-oxo-4,5-dihydro-1*H*-imidazol-2-yl]-*N′*-isopropylguanidine Derivatives (9a−e)[a]



[a] Reagents and conditions: (i) BrCN/AcOH, 4 h, 25 °C, 85%; (ii) Na, EtOH, 25 °C; (iii) $ClCH_2CONH_2/CH_3CN$, reflux, 4 h, 80%; (iv) **18**, K_2CO_3, 1-propanol/DMF, 100 °C, 24 h; (v) $(CH_3)_2CHNHCN/CH_3CH_2CH_2OH$, reflux, 24 h, 65%; (vi) $(RO)_2C{=}O$ or RCOCl, Et_3N, DMF, DMAP, 25 °C, 45−55%.

EXPERIMENTAL SECTION

A. Chemistry. Melting points were determined in open capillary tubes on an OptiMelt melting point apparatus (Standard Research Systems, U.S.) and are uncorrected. ^1H NMR and ^{13}C NMR spectra were recorded using Bruker Avance 300 and Bruker Avance 600 spectrometers (Bruker Instruments, Inc., Wilmington, DE). Chemical shifts are given in ppm (δ) relative to tetramethylsilane (TMS) as internal standard. Analytical thin-layer chromatography (TLC) was performed using HPLC-HLF normal phase 150 μm silica gel plates (Analtech, Newark, DE). Visualization of the developed chromatogram was performed with UV absorbance. Flash chromatography was conducted with silica gel 60 Å (200−400 mesh) from Sigma-Aldrich Co. Solvents and reagents obtained from commercial sources were used without purification, unless otherwise noted. Reactions were carried out under an inert atmosphere of nitrogen. Elemental analysis was 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. An LC/UV−vis/trap MS was employed for analysis of purity and chromophore properties. The system consisted of an Agilent 1100 series LC/UV−vis system online with a ThermoFinnigan (now Thermo Scientific; Waltham, MA) LCQ

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MS instrument equipped with an electrospray ionization (ESI) source. Samples were analyzed using shallow CH_3CN/1% $HCOOH/H_2O$ gradients at low flow rate. The purity of all final products was ≥95%.

*General Procedure for the Preparation of **11a**−**c**.* A mixture of 2-alkylaminoacetic acid (0.1 mol) and NH_4SCN (22.8 g, 0.3 mol) was heated at 140 °C for 6 h. The dark red solution was cooled. The solid cake formed was crushed with a spatula, washed with 40 mL of H_2O, and collected. The crystals were then washed successively with H_2O, 95% ethanol, and hexane to give 7.5 g (65%) of light tan crystals after drying. The products were determined to be at least 93−95% pure by LC/MS analytical method described in Experimental Section above and were used without further purification.

*1-Methyl-2-thioxoimidazolidin-4-one (**11a**).* Mp 222−224 °C. Yield: 45%. Light tan crystals. ^{1}H NMR (DMSO-d_6): δ 11.67 (s, 1H), 4.27 (s, 2H), 3.23 (s, 3H). ^{13}C NMR (DMSO-d_6): δ 181.5, 172.1, 55.1, and 32.7. MS (EI): m/z 130.0 $[M]^+$.

*1-Isopropyl-2-thioxoimidazolidin-4-one (**11b**).* Mp 158 °C. Yield: 55%. ^{1}H NMR ($CDCl_3$): δ 9.26 (s, 1H), 4.95 (m, 1H), 4.01 (s, 2H), 1.26 (d, 6H). ^{13}C NMR ($CDCl_3$): δ 179.8, 171.0, 48.3, 47.3, 19.6. MS (EI): m/z 158.0 $[M]^+$

*1-tert-Butyl-2-thioxoimidazolidin-4-one (**11c**).* Mp 119−120 °C. Yield: 40%. ^{1}H NMR ($CDCl_3$): δ 9.20 (s, 1H), 3.80 (s, 2H), 1.45 (s, 9H). ^{13}C NMR ($CDCl_3$): δ 180.9, 170.7, 47.1, 29.4. MS (ESI): m/z 172.2 $[M]^+$.

*General Procedure for the Preparation of **12a**,**b**.* A solution of 2-thiohydantoin **11** (5 mmol) in 5 mL of DMF at 0 °C was treated with CH_3I (0.8 mL, 10.6 mmol). The solution was warmed slowly to 35 °C over 30 min and stirred for an additional 5 h. The precipitated compound was collected and washed with ethanol followed by hexanes. The products were determined to be at least 93−95% pure by LC/MS described in Experimental Section above and were used without further purification.

*1-Methyl-2-methylsulfanyl-1,5-dihydroimidazol-4-one HI Salt (**12a**).* Yield: 88%. Mp 226−228 °C. White solid. ^{1}H NMR (D_2O): δ 4.70 (s, 2H), 3.25 (s, 3H), 2.73 (s, 3H). ^{13}C NMR (D_2O): δ 176.6, 174.0, 57.7, 34.8, 15.2. MS (EI): m/z 144.0 $[M]^+$.

*1-Isopropyl-2-methylsulfanyl-1,5-dihydroimidazol-4-one HI Salt (**12b**).* Yield: 90%. Mp 198 °C. White solid. ^{1}H NMR (D_2O): δ 4.69 (s, 2H), 4.19 (m, 1H), 2.66 (s, 3H), 1.21 (s, 3H), 1.19 (s, 3H). ^{13}C NMR (D_2O): δ 175.1, 173.6, 51.4, 18.9, 14.1. MS (EI): m/z 172.0 $[M]^+$.

*General Procedure for the Preparation of **13a**,**b**.* To the water solution of **12a**,**b** salt (1.3 g) was added saturated Na_2CO_3 solution at ice-cold temperature until the pH reached ∼9−10. The reaction mixture was extracted with CH_2Cl_2 three times. The organic solution was dried over Na_2SO_4 and evaporated to dryness under reduced pressure. The residue was purified by a silica gel column using hexane/EtOAc (4:1 v/v) to give product **13**.

*1-Methyl-2-methylsulfanyl-1,5-dihydroimidazol-4-one (**13a**).* Yield: 98%. Mp 118.0−120 °C. White solid. ^{1}H NMR ($CDCl_3$): δ 3.99 (s, 2H), 3.15 (s, 3H), 2.66 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 186.1, 184.9, 57.5, 32.8, 15.0. MS (EI): m/z 144.0 $[M]^+$.

*1-Isopropyl-2-methylsulfanyl-1,5-dihydroimidazol-4-one (**13b**).* Yield: 98%. Colorless oil. ^{1}H NMR ($CDCl_3$): δ 4.05 (m, 1H), 3.91 (s, 2H), 2.67 (s, 3H), 1.28 (s, 3H), 1.26 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 184.9, 184.5, 50.3, 20.7, 14.8. MS (EI): m/z 172.0 $[M]^+$.

*General Procedure for the Preparation of Compounds **15a**−**c**. Method A.* 2-Methylthioimidazolin-4-one **13b** (0.86 g, 5 mmol) in MeOH was added to 8 mL of 7.0 M ammonia in MeOH. The mixture was refluxed at 65 °C for 5 h. The MeOH was removed under reduced pressure. The crude product was purified by first suspending in 20 mL of CH_2Cl_2. The product was collected by filtration, washed twice with CH_2Cl_2, and dried to give the white product in 80−88% yield.

Method B. To alkylcyanamide (RNHCN, where R = $-CH_3$, $-CH(CH_3)_2$, or $-C(CH_3)_3$, 16.5 mmol) in 15 mL of absolute EtOH was added sodium metal (0.38 g, 16.5 mmol) in small portions. The mixture was stirred at room temperature until the sodium metal was consumed. The solvent was evaporated to dryness under reduced pressure. The solid was suspended in 20 mL of CH_3CN. To the suspension chloroacetamide (1.7 g, 18.1 mmol) was added and the mixture was refluxed for 5 h. The white precipitates were collected after cooling, washed successively with H_2O and acetone, and dried to give the product in 80−85% yield. The products were used without further purification.

*2-Amino-1-methyl-1,5-dihydroimidazol-4-one (**15a**).* **15a** was prepared by method A. Mp 295−296 °C. White solid. ^{1}H NMR (D_2O): δ 4.70 (s, 2H), 3.20 (s, 3H). ^{13}C NMR (D_2O): δ 186.3, 184.0, 57.7, 34.8. MS (EI): m/z 113.0 $[M]^+$.

*2-Amino-1-isopropyl-1,5-dihydroimidazol-4-one (**15b**).* **15b** was prepared by methods A and B. Mp 225−226 °C. White solid. ^{1}H NMR (MeOD): δ 4.07 (q, J = 6.72 Hz, 1H), 3.90 (s, 1H), 1.24 (s, 3H), 1.23 (s, 3H). ^{13}C NMR (CD_3OD): 187.0, 168.9, 45.0, 21.2, 19.0. IR (neat): 3308, 1658, and 1497 cm^{-1}. MS (EI): m/z 141.0 $[M]^+$.

*2-Amino-1-tert-butyl-1,5-dihydroimidazol-4-one (**15c**).* **15c** was prepared by method B. Mp 230−231 °C. Yield: 50%. White solid. ^{1}H NMR (DMSO-d_6): δ 7.02 (s, 2H), 3.87 (s, 2H), 1.34 (s, 9H). ^{13}C NMR (DMSO-d_6): δ 184.3, 169.6, 53.9, 53.3, 28.4. IR (neat): 3300, 1665, 1470, and 1415 cm^{-1}. MS (ESI): m/z 156.0 $[M + 1]^+$.

*General Procedure for the Preparation of **8a**,**b**.* To the solution of free base 2-methylthio-imidazol-4-one (**13**, 7.0 mmol) in 10 mL of EtOH was added 3,4-dichlorophenylguanidine (1.7 g, 8.3 mmol). The reaction mixture was heated at 80 °C for 48 h. The pale solid precipitates were collected, washed with CH_3OH, and dried to afford the product as a pale white solid.

*N-(3,4-Dichlorophenyl)-N′-(1-methyl-4-oxo-4,5-dihydro-1H-imidazol-2-yl)guanidine (**8a**).* Yield 70%. Mp 256.6−258.5 °C. White solid. ^{1}H NMR (DMSO-d_6): δ 9.50 (s, 1H), 8.04 (d, J = 2.45 Hz, 1H), 7.58 (d, J = 8.76 Hz, 1H), 7.35 (dd, J = 8.74 Hz, J = 2.45 Hz, 1H), 3.75 (s, 2H), 3.02 (s, 3H). ^{13}C NMR (DMSO-d_6): δ 185.1, 174.2, 157.7, 139.1, 131.2, 130.9, 125.2, 123.1, 121.6, 54.2, 30.6. IR (neat): 3148, 1878, 1550, and 1470 cm^{-1}. MS (EI): m/z 299.0 $[M]^+$. Anal. ($C_{11}H_{11}Cl_2N_5O$) C, H, N, Cl.

*N-(3,4-Dichlorophenyl)-N′-(1-isopropyl-4-oxo-4,5-dihydro-1H-imidazol-2-yl)guanidine (**8b**).* Yield 75%. Mp 231−232 °C. White solid. ^{1}H NMR (DMSO-d_6): δ 9.45 (s, 1H), 8.06 (d, J = 2.36 Hz, 1H), 7.54 (d, J = 8.75 Hz, 1H), 7.29 (dd, J = 8.75 Hz, J = 2.36 Hz, 1H), 4.39 (m, 1H), 3.70 (s, 2H), 1.16 (s, 3H), 1.14 (s, 3H). ^{13}C NMR (DMSO-d_6): δ 185.1, 173.1, 157.6, 139.2, 131.2, 130.8, 125.1, 123.1, 121.4, 47.4, 44.2, 20.7. IR (neat): 3150, 1680, 1600, and 1475 cm^{-1}. MS (EI): m/z 327.0 $[M]^+$. Anal. ($C_{13}H_{15}Cl_2N_5O$) C, H, N, Cl.

*Synthesis of N-(3,4-Dichlorophenyl)-N′-(1-tert-butyl-4-oxo-4,5-dihydro-1H-imidazol-2-yl)guanidine (**8c**).* A mixture consisting of 1-*tert*-butyl-2-aminoimidazolin-4-one (**15c**) (3.0 g, 19.3 mmol) and 3,4-dichlorophenylcyanamide (3.7 g, 20 mmol) in 1-propanol (30 mL) was heated at 100 °C for 24 h. After cooling, the solution was filtered and the solvent was removed under reduced pressure. The crude product was purified by a silica gel column using CH_2Cl_2/MeOH (10:1 v/v) as eluent and recrystallized from MeOH to give 3.9 g of white crystals. Mp 214−216 °C. ^{1}H NMR (DMSO-d_6): δ 9.40 (s, 1H), 7.88 (s, 1H), 7.54 (d, J = 8.65 Hz, 1H), 7.20 (dd, J = 8.65 Hz, J = 2.40 Hz, 1H), 3.87 (s, 2H), 1.36 (s, 9H). ^{13}C NMR (DMSO-d_6): δ 184.3, 173.5, 157.3, 138.6, 131.3, 130.8, 126.0, 125.0, 124.8, 122.9, 122.8, 54.4, 51.6, 28.7. IR (neat): 3367.4, 1660.9, 1466.3, and 407.2 cm^{-1}. MS (ESI): m/z 341.97 $[M + 1]^+$. Anal. ($C_{14}H_{17}Cl_2N_5O$) C, H, N, Cl.

*General Procedure for the Preparation of Compounds **8d**−**i**.* To a mixture of **8a**, **8b**, or **8c** (5.40 mmol) and DMF (10 mL) were added DMAP (0.3 g, 2.67 mmol) and Et_3N (0.5 mL), followed by addition of dicarbonate (5.61 mmol) or acyl chloride (5.61 mmol). The reaction mixture was stirred at room temperature for 12 h and evaporated to dryness. The solid was dissolved in CH_2Cl_2 (30 mL), filtered, and the

filtrate was washed with water, dried over Na_2SO_4, and evaporated to dryness to give the crude product. The crude product was purified by a silica gel column and eluted with EtOAc/hexane (4:1 v/v) to give the desired product.

*N-(tert-Butoxycarbonyl)-N′-(3,4-dichlorophenyl)-N″-(1-methyl-4-oxo-4,5-dihydro-1H-imidazol-2-yl)guanidine (**8d**).* The title compound was prepared according to the general procedure using **8a** and di-*tert*-butyl dicarbonate as acylating agent to give 45% yield of the product as white crystals. Mp 234−235 °C. ^{1}H NMR ($CDCl_3$): δ 12.80 (s, 1H), 10.81 (s, 1H), 8.00 (d, J = 2.46 Hz, 1H), 7.43 (d, J = 8.70 Hz, 1H), 7.39 (dd, J = 8.70 Hz, J = 2.46 Hz, 1H), 3.86 (s, 2H), 3.14 (s, 3H), 1.54 (s, 9H). ^{13}C NMR ($CDCl_3$): δ 184.3, 172.4, 153.8, 152.9, 136.0, 132.5, 130.2, 128.6, 124.5, 121.6, 84.6, 54.2, 30.8, 28.0. IR (neat): 3105, 1670, 1555, and 1470 cm^{-1}. MS (EI): m/z 399 $[M]^+$. Anal. ($C_{16}H_{19}Cl_2N_5O_3$) C, H, N, Cl.

*N-(tert-Butoxycarbonyl)-N′-(3,4-dichlorophenyl)-N″-(1-isopropyl-4-oxo-4,5-dihydro-1H-imidazol-2-yl)guanidine (**8e**).* Compound **8e** was prepared by reaction of **8b** with di-*tert*-butyl dicarbonate. Yield 50%. White crystals. Mp 152−153 °C. ^{1}H NMR ($CDCl_3$): δ 12.73 (s, 1H), 10.81 (s, 1H), 8.05 (d, J = 2.37 Hz, 1H), 7.43 (d, J = 8.67 Hz, 1H), 7.24 (dd, J = 8.67 Hz, J = 2.37 Hz, 1H), 4.51 (m, 1H), 3.80 (s, 2H), 1.54 (s, 9H), 1.24 (s, 3H), 1.22 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 184.68, 171.30, 153.88, 152.76, 136.11, 132.50, 130.22, 128.50, 124.50, 121.45, 84.58, 47.26, 44.83, 28.09, 20.79. IR (neat): 3100, 1658, 1574, and 1450 cm^{-1}. MS (EI): m/z 427.0 $[M]^+$. Anal. ($C_{18}H_{23}Cl_2N_5O_3$) C, H, N, Cl.

*N-(tert-Butoxycarbonyl)-N′-(3,4-dichlorophenyl)-N″-(1-tert-butyl-4-oxo-4,5-dihydro-1H-imidazol-2-yl)guanidine (**8f**).* Compound **8f** was prepared by treatment of **8c** with di-*tert*-butyl dicarbonate. Yield 55%. White crystals. Mp 168−169 °C. ^{1}H NMR ($CDCl_3$): δ 13.02 (s, 1H), 10.64 (s, 1H), 7.76 (d, J = 2.37 Hz, 1H), 7.43 (d, J = 8.61 Hz, 1H), 7.14 (dd, J = 8.61 Hz, J = 2.37 Hz, 1H), 3.91 (s, 2H), 1.50 (s, 9H), 1.35 (s, 9H). ^{13}C NMR ($CDCl_3$): δ 183.27, 171.59, 154.35, 153.25, 135.77, 132.58, 130.27, 129.63, 126.90, 123.67, 84.21, 55.30, 51.47, 28.84, 28.10. IR (neat): 3100, 1650, 1575, and 1460 cm^{-1}. MS (ESI): m/z 442.3 $[M + 1]^+$. Anal. ($C_{19}H_{25}Cl_2N_5O_3$) C, H, N, Cl.

*N-(Ethoxycarbonyl)-N′-(3,4-dichlorophenyl)-N″-(1-isopropyl-4-oxo-4,5-dihydro-1H-imidazol-2-yl)guanidine (**8g**).* The title compound was prepared by treatment of **8b** with ethyl chloroformate. Yield: 48%. Mp 279.3−280.0 °C. White crystals. ^{1}H NMR ($CDCl_3$): δ 13.1 (br s, 1H), 10.7 (s, 1H), 8.03 (d, J = 2.3 Hz, 1H), 7.42 (d, J = 8.6 Hz, 1H), 7.20 (dd, J = 8.60 Hz, J = 2.3 Hz, 1H), 4.53 (m, 1H), 4.26 (q, J = 7.0 Hz, 2H), 3.80 (s, 2H), 1.37 (t, J = 7.0 Hz, 3H), 1.25 (d, J = 6.5 Hz, 6H). ^{13}C NMR ($CDCl_3$): δ 184.8, 169.9, 155.6, 153.5, 136.1, 132.5, 130.2, 128.6, 124.6, 121.4, 62.9, 47.1, 44.9, 20.7, 14.2. MS (ESI): m/z 400 $[M + 1]^+$. Anal. ($C_{16}H_{19}Cl_2N_5O_3$) C, H, N, Cl.

*N-(3,4-Dichlorophenyl)-N′-(1-isopropyl-4-oxo-4,5-dihydro-1H-imidazol-2-yl)-N″-(trimethylacetyl)guanidine (**8h**).* Compound **8h** was prepared by treatment of **8b** with trimethylacetyl chloride. Yield: 45%. Mp 189−190 °C. White crystals. ^{1}H NMR ($CDCl_3$): δ 14.01 (s, 1H), 11.59 (s, 1H), 8.10 (d, J = 2.40 Hz, 1H), 7.40 (d, J = 8.70 Hz, 1H), 7.24 (dd, J = 8.67 Hz, J = 2.40 Hz, 1H), 4.54 (q, J = 7.50 Hz, 1H), 3.81 (s, 2H), 1.36 (s, 9H), 1.26 (s, 3H), 1.24 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 184.0, 182.6, 171.6, 153.0, 136.0, 132.5, 130.3, 128.7, 124.5, 121.4, 47.4, 44.9, 41.0, 27.0, 20.7. IR (neat): 3100, 1670, 1558, and 1468 cm^{-1}. MS (ESI): m/z 412.22 $[M + 1]^+$. Anal. ($C_{18}H_{23}Cl_2N_5O_2$) C, H, N, Cl

*N-(2-Ethylbutyryl)-N′-(3,4-dichlorophenyl)-N″-(1-isopropyl-4-oxo-4,5-dihydro-1H-imidazol-2-yl)guanidine (**8i**).* Compound **8i** was prepared by treatment of **8b** with 2-ethylbutyryl chloride. Yield: 32%. White solid. Mp 142.6−143.8 °C. ^{1}H NMR ($CDCl_3$): δ 13.9 (br s, 1H), 11.5 (s, 1H), 8.12 (br s, 1H), 7.43 (d, J = 8.6 Hz, 1H), 7.24 (d, J = 8.6 Hz, 1H), 4.58 (m, 1H), 3.82 (s, 2H), 2.35 (m, 1H), 1.68 (m, 4H), 1.26 (d, J = 6.7 Hz, 6H), 0.97 (t, J = 7.3 Hz, 6H). ^{13}C NMR ($CDCl_3$): δ 184.2, 179.8, 171.6, 152.6, 135.9, 132.6, 130.3, 128.7, 124.5, 121.4, 52.3, 47.4, 44.9, 25.0, 20.8, 11.6. MS (ESI): 426 m/z $[M + 1]^+$. Anal. ($C_{19}H_{25}Cl_2N_5O_2$) C, H, N, Cl.

*General Procedure for the Preparation of Compounds **8j**−**n**.* A mixture of 2-aminoimidazolin-4-one (**15**, 14.2 mmol), 2-methylisothiourea **18** (20.0 mmol), and K_2CO_3 (2.0 g, 14.2 mmol) in 1-propanol (20 mL) was heated at 110 °C for 48 h and filtered to remove the K_2CO_3. The solvent was evaporated to dryness, and the residue was suspended in 10 mL of CH_2Cl_2. The solid K_2CO_3 was removed by filtration, and the filtrate was evaporated to dryness. The crude product was purified with a silica gel column using CH_2Cl_2/MeOH (10:1 v/v) as eluent to give the final product which was further purified by recrystallization from Et_2O and EtOAc.

*N-(3,4-Dichlorophenyl)-N′-isopropyl-N″-(1-isopropyl-4-oxo-4,5-dihydro-1H-imidazol-2-yl)guanidine (**8j**).* The title compound was prepared from **15b** and **18a**. Yield: 75%. Mp 195−196 °C. White crystals. ^{1}H NMR ($CDCl_3$): δ 11.83 (br, 1H), 7.47 (d, J = 8.50 Hz, 1H), 7.36 (d, J = 2.36 Hz, 1H), 7.04 (dd, J = 8.50 Hz, J = 2.36 Hz, 1H), 4.57 (q, J = 6.90 Hz, 1H), 4.11 (br, 1H), 3.70 (s, 2H), 1.24 (s, 3H), 1.23 (s, 3H), 1.21 (s, 3H), 1.19 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 188.1, 172.3, 155.5, 135.0, 142.1, 130.5, 128.4, 123.3, 46.9, 43.8, 22.7, 20.6. IR (neat): 3300, 1680, 1660, and 1470 cm^{-1}. MS (EI): m/z 369.0 $[M]^+$. Anal. ($C_{16}H_{21}Cl_2N_5O$) C, H, N, Cl.

*N-(tert-Butyl)-N′-(3,4-dichlorophenyl)-N″-(1-isopropyl-4-oxo-4,5-dihydro-1H-imidazol-2-yl)guanidine (**8k**).* Compound **8k** was prepared from **15b** and **18b**. Yield: 75%. Mp 178−179 °C. White crystals. ^{1}H NMR ($CDCl_3$): δ 11.95 (s, 1H), 7.48 (d, J = 8.53 Hz, 1H), 7.33 (d, J = 2.1 Hz, 1H), 7.04 (dd, J = 8.53 Hz, J = 2.1 Hz, 1H), 4.70 (br, 1H), 4.61 (q, J = 6.70 Hz, 1H), 3.74 (s, 2H), 1.43 (s, 9H), 1.23 (s, 3H), 1.21 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 185.5, 171.7, 155.7, 136.0, 133.7 131.5, 130.5, 127.4, 124.7, 52.6, 46.8, 44.1, 29.5, 20.7. IR (neat): 3300, 1692, 1564, and 1506 cm^{-1}. MS (EI]: m/z 383.0 $[M^+]$. Anal. ($C_{17}H_{23}Cl_2N_5O$) C, H, N, Cl.

*N-(Ethyl)-N′-(3,4-dichlorophenyl)-N″-(1-isopropyl-4-oxo-4,5-dihydro-1H-imidazol-2-yl)guanidine (**8l**).* Compound **8l** was prepared from **15b** and **18c**. Yield: 70%. Mp 178−179 °C. White crystals. ^{1}H NMR ($CDCl_3$): δ 11.78 (s, 1H), 7.31−7.41 (m, 2H), 7.06 (s, 1H), 5.13 (s, 1H), 4.57 (s, 1H), 3.69 (s, 2H), 3.43 (q, J = 6.63 Hz, 2H), 1.18 (m, 9H). ^{13}C NMR ($CDCl_3$): δ 185.7, 171.8, 156.2, 135.2, 134.0, 131.4, 130.4, 128.1, 126.5, 46.8, 43.9, 36.6, 20.6, 14.9. IR (neat): 3200, 1686, 1507, and 1439 cm^{-1}. MS (ESI): m/z 356.12 $[M + 1]^+$. Anal. ($C_{15}H_{19}Cl_2N_5O$) C, H, N, Cl.

*N-(Adamantyl)-N′-(3,4-dichlorophenyl)-N″-(1-isopropyl-4-oxo-4,5-dihydro-1H-imidazol-2-yl)guanidine (**8m**).* Compound **8m** was prepared from **15b** and **18d**. Yield: 75%. Mp 197−198 °C. White crystals. ^{1}H NMR ($CDCl_3$): δ 11.95 (br, 1H), 7.47 (d, J = 8.53 Hz, 1H), 7.34 (d, J = 2.37 Hz, 1H), 7.05 (dd, J = 8.53 Hz, J = 2.37 Hz, 1H), 4.57 (m, 2H), 3.74 (s, 2H), 2.08 (m, 9H), 1.66 (m, 6H), 1.26 (s, 3H), 1.22 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 185.5, 171.5, 155.4, 136.0, 133.7, 131.4, 130.5, 127.4, 124.7, 53.2, 46.9, 44.2, 42.2, 36.3, 29.4, 20.8. IR (neat): 3200, 1702, 1565, and 1503 cm^{-1}. MS (EI): m/z 461.0 $[M]^+$. Anal. ($C_{23}H_{29}Cl_2N_5O$) C, H, N, Cl.

*N-(Isopropyl)-N′-(3,4-dichlorophenyl)-N″-(1-methyl-4-oxo-4,5-dihydro-1H-imidazol-2-yl)guanidine (**8n**).* The title compound was prepared from **15a** and **18a**. Yield: 70%. Mp 208−210 °C. White crystals. ^{1}H NMR (DMSO-d_6): δ 10.11 (br, 1H), 8.86 (s, 1H), 7.88 (s, 1H), 7.59 (d, J = 8.64 Hz, 1H), 7.46 (m, 1H), 4.09 (br, 1H), 3.68 (s, 2H), 2.93 (s, 3H), 1.22 (s, 3H), 1.20 (s, 3H). ^{13}C NMR (DMSO-d_6): δ 184.5, 173.0, 155.2, 141.0, 130.5, 125.8, 124.1, 53.9, 43.4, 30.4, 23.1. IR (neat): 1690, 1514, 1460, and 302 cm^{-1}. MS (ESI): m/z 342.06 $[M + 1]^+$. Anal. ($C_{14}H_{17}Cl_2N_5O$) C, H, N, Cl.

*Synthesis of 2,2-Dimethylpropionic Acid 2-[N′-(3,4-Dichlorophenyl)-N″-(2,2-dimethylpropionyl)guanidino]-1-isopropyl-1H-imidazol-4-yl Ester (**14**).* To a mixture of starting material 1-isopropyl-4-oxoimidazolinoguanidine **8b** (0.55 g, 1.68 mmol) in $CHCl_3$ (10 mL) were added

DMAP (0.41 g, 3.36 mmol) and Et_3N (0.5 g, 5.0 mmol), followed by addition of trimethylacetyl chloride (0.42 g, 3.36 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 2 h. The solvent was evaporated to dryness and the product was purified by silica gel column using hexane/EtOAc (10:1 v/v) to give 0.80 g (95%) of compound **14** as white crystals. Mp 128−129 °C. ^{1}H NMR (CDCl3): δ 13.28 (s, 1H), 10.93 (s, 1H), 8.28 (d, J = 2.43 Hz, 1H), 7.33 (d, J = 8.70 Hz, 1H), 7.23 (dd, J = 8.70 Hz, J = 2.43 Hz, 1H), 6.65 (s, 1H), 4.76 (q, J = 6.01 Hz, 1H), 1.44 (s, 3H), 1.42 (s, 3H), 1.35 (s, 9H), 1.33 (s, 9H). ^{13}C NMR ($CDCl_3$): δ 181.3, 175.6, 145.3, 142.7, 142.6, 137.9, 132.3, 130.0, 125.9, 122.3, 119.6, 97.6, 46.2, 40.8, 39.0, 27.0, 26.5, 22.6. MS (ESI): m/z 496.25 $[M + 1]^+$. Anal. ($C_{23}H_{31}Cl_2N_5O_3$) C, H, N, Cl.

*Synthesis of N-[1-(3,4-Dichlorophenyl)-4-oxo-4,5-dihydro-1H-imidazol-2-yl]-N′-isopropylguanidine (**9a**).* A mixture of 2-amino-3-(3,4-dichlorophenyl)imidazolin-4-one (**24**, 3.0 g, 12.3 mmol) and isopropylcyanamide (2.1 g, 24.6 mmol) in 1-propylanol (30 mL) was heated at 110 °C for 48 h, filtered, and the solvent was removed under reduced pressure. The product was purified by a silica gel column to give 1.7 g (45%) of the product which was recrystallized from MeOH. Mp 260−262 °C. ^{1}H NMR (DMSO-d_6): δ 8.68 (br, 1H), 8.37 (br, 1H), 7.95 (m, 1H), 7.52 (m, 2H), 7.14 (s, 1H), 4.03 (s, 2H), 4.01 (q, J = 6.0 Hz, 1H), 1.19 (s, 3H), 1.17 (s, 3H). ^{13}C NMR (DMSO-d_6): δ 182.8, 171.1, 159.2, 139.4, 131.1, 130.4, 124.5, 122.3, 121.7, 120.8, 120.1, 52.3, 43.1, 11.5. IR (neat): 3414, 1703, 1654, and 1476 cm^{-1}. MS (ESI): m/z 328.06 $[M + 1]^+$. Anal. ($C_{13}H_{15}Cl_2N_5O$) C, H, N, Cl.

*General Procedure for the Preparation of **9b**−**e**.* To a mixture of compound **9a** (1.80 g, 5.40 mmol) and DMF (10 mL) was added DMAP (0.3 g, 2.67 mmol) and Et_3N (0.5 mL), followed by addition of dicarbonate (5.61 mmol) or acyl chloride (5.61 mmol). The reaction mixture was stirred at 30 °C for 12 h, and the solvent was evaporated to dryness. The residue was suspended in CH_2Cl_2 (30 mL) and filtered to remove the unreacted starting material. The filtrate was evaporated to dryness and purified by a silica gel column, eluting with hexane/ethyl acetate (1:1 v/v) mixed solvent. The product was recrystallized from hexane/EtOAc.

*N-(tert-Butoxycarbonyl)-N′-[1-(3,4-dichlorophenyl)-4-oxo-4,5-dihydro-1H-imidazol-2-yl]-N″-isopropylguanidine (**9b**).* Compound **9b** was prepared from **9a** and di-*tert*-butyl dicarbonate. Yield: 48%. Mp 246−248 °C. White crystals. ^{1}HNMR ($CDCl_3$): δ 12.41 (s, 1H), 8.83 (d, J = 6.27 Hz, 1H), 8.09 (d, J = 2.58 Hz, 1H), 7.39 (d, J = 8.61 Hz, 1H), 7.17 (dd, J = 8.61 Hz, J = 2.58 Hz, 1H), 4.24−4.26 (m, 1H), 4.20 (s, 2H), 1.52 (s, 9H), 1.31 (s, 3H), 1.30 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 182.4, 170.5, 154.6, 153.4, 137.6, 132.5, 130.1, 127.5, 123.5, 119.5, 84.1, 52.4, 44.0, 28.0, 22.4. IR (neat): 1723, 1606, 1505, and 1152 cm^{-1}. MS (ESI): m/z 430.0 $[M + 1]^+$. Anal. ($C_{18}H_{23}Cl_2N_5O_3$) C, H, N, Cl.

*N-(Ethoxycarbonyl)-N′-[1-(3,4-dichlorophenyl)-4-oxo-4,5-dihydro-1H-imidazol-2-yl]-N″-isopropylguanidine (**9c**).* Compound **9c** was prepared from **9a** and ethyl chloroformate. Yield: 50%. Mp 193.5−195 °C. White crystals. ^{1}H NMR ($CDCl_3$): ^{1}H NMR ($CDCl_3$): δ 12.9 (br, 1H), 8.76 (br, 1H), 8.10 (d, J = 2.5 Hz, 1H), 7.42 (d, J = 8.8 Hz, 1H), 7.20 (dd, J = 2.5 Hz, J = 8.8 Hz, 1H), 4.26−4.25 (m, 5H), 1.35 (m, 9H). ^{13}C NMR ($CDCl_3$): δ 182.2, 170.6, 154.4, 154.3, 137.6, 132.5, 130.2, 127.7, 123.6, 119.6, 62.9, 52.5, 44.2, 22.4, 14.2. MS (ESI): m/z 400.05 $[M + 1]^+$. Anal. ($C_{16}H_{19}Cl_2N_5O_3$) C, H, N, Cl.

*N-[1-(3,4-Dichlorophenyl)-4-oxo-4,5-dihydro-1H-imidazol-2-yl]-N′-isopropyl-N″-(trimethylacetyl)guanidine (**9d**).* Compound **9d** was prepared from compound **9a** and trimethylacetyl chloride. Yield: 50%. White crystals. Mp 182−183 °C. ^{1}H NMR ($CDCl_3$): 13.68 (s, 1H), 9.63 (s, 1H), 8.12 (d, J = 2.37 Hz, 1H), 7.42−7.39 (d, J = 8.76 Hz, 1H), 7.21−7.18 (dd, J = 8.76 Hz, J = 2.37 Hz, 1H), 4.29 (m, 1H), 4.21 (s, 2H), 1.34−1.12 (m, 15H). ^{13}C NMR ($CDCl_3$): 182.1, 181.7, 170.5, 158.1, 154.9, 137.5, 132.5, 130.2, 127.7, 123.5, 119.5, 118.2, 52.5, 44.1, 26.9. IR (neat): 1731, 1612, 1571, and 1484 cm^{-1}. MS (ESI): m/z 412.40 $[M + 1]^+$. Anal. ($C_{18}H_{23}Cl_2N_5O_2$) C, H, N, Cl.

*N-[1-(3,4-Dichlorophenyl)-4-oxo-4,5-dihydro-1H-imidazol-2-yl]-N′-isopropyl-N″-(2-ethtylbutyryl)guanidine (**9e**).* Compound **9e** was prepared from **9a** and 2-ethylbutyryl chloride. Yield: 45%, white crystals. Mp 149.3−150.2 °C. ^{1}H NMR ($CDCl_3$): δ 13.5 (s, 1H), 9.59 (d, J = 6.4 Hz, 1H), 8.12 (d, J = 2.3 Hz, 1H), 7.42 (d, J = 8.7 Hz, 1H), 7.20 (dd, J = 8.70 Hz, J = 2.3 Hz, 1H), 4.53 (m, 1H), 4.27 (m, 3H), 2.30 (m, 1H), 1.67 (m, 4H), 1.33 (d, J = 6.5 Hz, 6H), 0.96 (t, J = 7.4 Hz, 6H). ^{13}C NMR: ($CDCl_3$) δ 181.9, 179.4, 170.6, 154.5, 137.5, 132.6, 130.2, 127.8, 123.6, 119.6, 52.5, 52.2, 44.1, 25.0, 22.4, 11.7. MS (ESI): m/z 426.0 $[M + 1]^+$. Anal. ($C_{19}H_{25}Cl_2N_5O_2$) C, H, N, Cl.

*General Procedure for the Preparation of **17a**−**d**.* 3,4-Dichlorophenyl isothiocyanate (**16**, 5.0 g, 24.5 mmol) was added dropwise to an appropriate amine (27 mmol) in CH_2Cl_2 (15 mL). The mixture was stirred for 18 h at room temperature, and the solvent was then removed under reduced pressure. The solid product was suspended in Et_2O (20 mL), collected, washed several times with fresh Et_2O, and dried.

*1-(3,4-Dichlorophenyl)-3-isopropylthiourea (**17a**).* The title compound was prepared by treatment of 3,4-dichlorophenyl isothiocyanate **16** with isopropylamine as described above. Yield: 86%. Mp 145−146 °C. White solid. ^{1}H NMR ($CDCl_3$): δ 8.44 (s, 1H), 7.47 (d, J = 8.55 Hz, 1H), 7.34 (d, J = 2.65 Hz, 1H), 7.08 (dd, J = 8.55 Hz, J = 2.65 Hz, 1H), 5.86 (s, 1H), 4.53 (q, J = 6.28 Hz, 1H), 1.26 (s, 3H), 1.24 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 179.0, 135.8, 133.9, 131.6, 130.8, 126.5, 124.0, 47.5, 22.3. IR (neat): 3308, 1583, 1538, and 1474 cm^{-1}. MS (EI): m/z 264.0 $[M]^+$.

*1-tert-Butyl-3-(3,4-dichlorophenyl)thiourea (**17b**).* Title compound was prepared by treatment of **16** with *tert*-butylamine. Yield: 80%. Mp 157−158 °C. White solid. ^{1}H NMR ($CDCl_3$): δ 8.07 (s, 1H), 7.48 (d, J = 8.54 Hz, 1H), 7.35 (s, 1H), 7.09 (d, J = 8.39 Hz, 1H), 6.07 (s, 1H), 1.52 (s, 9H). ^{13}C NMR ($CDCl_3$): δ 179.1, 136.4, 133.7, 131.4, 130.3, 126.3, 123.9, 54.3, 28.9. IR (neat): 3205, 1558, 1532, and 1473 cm^{-1}. MS (EI): m/z 276.0 $[M]^+$.

*1-(3,4-Dichlorophenyl)-3-ethylthiourea (**17c**).* The title compound was prepared from **16** and ethylamine according to the general procedure described above. Yield: 87%. Mp 113−114 °C. White solid. ^{1}H NMR ($CDCl_3$): δ 8.54 (s, 1H), 7.46 (d, J = 8.54 Hz, 1H), 7.28 (d, J = 2.1 Hz, 1H), 7.12 (dd, J = 8.54 Hz, J = 2.1 Hz, 1H), 6.09 (br, 1H), 3.65 (q, J = 6.56 Hz, 2H), 1.21 (t, J = 2.75 Hz, 3H). ^{13}C NMR ($CDCl_3$): δ 180.1, 135.9, 133.7, 131.5, 130.8, 126.6, 124.2, 40.3, 14.2. IR (neat): 3119, 1631, 1535, and 1473 cm^{-1}. MS (EI): m/z 248.0 $[M]^+$.

*1-(Adamantan-1-yl)-3-(3,4-dichlorophenyl)thiourea (**17d**).* The title compound was prepared from **16** and adamantylamine. Yield: 87%. Mp 179−180 °C. White solid. ^{1}H NMR ($CDCl_3$): δ 9.44 (s, 1H), 7.97 (s, 1H), 7.47 (d, J = 7.76 Hz, 1H), 7.34 (s, 1H), 7.31 (d, J = 8.73 Hz, 1H), 2.20 (m, 6H), 2.02 (m, 3H), 1.61 (m, 6H). ^{13}C NMR ($CDCl_3$): δ 178.8, 140.4, 130.7, 130.3, 125.2, 124.1, 122.9, 54.0, 41.0, 40.5, 40.2, 39.9, 39.6, 39.4, 36.4, 29.4. IR (neat): 3119, 1540, 1500, and 1460 cm^{-1}. MS (EI): m/z 354.0 $[M]^+$.

*General Procedure for the Preparation of **18a**−**d**.* Methyl iodide (3.2 g, 22.5 mmol) was added dropwise to thiourea **17** (15.0 mmol) in acetone (50 mL) solution. The mixture was stirred at 60 °C for 4 h, and the solvent was evaporated to dryness under the reduced pressure. The product was suspended in Et_2O, collected, and washed with fresh ether. The white solid was dissolved in 50 mL of H_2O and THF (1:1 v/v) mixed solvent. The solution was basified with 1.0 M K_2CO_3 solution to pH 10 and extracted 3 times with 50 mL of CH_2Cl_2. The extracts were combined, dried over Na_2SO_4, and evaporated to dryness to give the product **18** in over 96% yield. The products are pure enough to be used without further purification.

*1-(3,4-Dichlorophenyl)-3-isopropyl-2-methylisothiourea (**18a**).* Yield: 98%. Pale yellow oil. ^{1}H NMR ($CDCl_3$): δ 7.29 (d, J = 8.45 Hz, 1H), 7.00 (d, J = 2.37 Hz, 1H), 6.72 (dd, J = 8.45 Hz, J = 2.39 Hz, 1H), 4.29 (m, 1H), 4.02(m, 1H), 2.06 (s, 3H), 1.22 (s, 3H), 1.20 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 149.5, 132.3, 130.4, 125.4, 124.1, 121.9, 44.7, 22.9, 14.1. MS (EI): m/z 278 $[M]^+$.

*1-tert-Butyl-3-(3,4-dichlorophenyl)-2-methylisothiourea (**18b**).* Yield: 99%. Colorless oil. ^{1}H NMR ($CDCl_3$): δ 7.29 (d, J = 8.51 Hz, 1H), 7.00 (d, J = 2.4 Hz, 1H), 6.73 (dd, J = 8.50 Hz, J = 2.4 Hz, 1H), 4.40 (s, 1H), 2.14 (s, 3H), 1.41 (s, 9H). ^{13}C NMR ($CDCl_3$): δ 150.3, 149.3, 132.0, 130.1, 125.0, 123.8, 121.7, 53.3, 28.7. IR (neat): 2995, 1584, 1475, and 1292 cm^{-1}. MS (EI): m/z 290.0 $[M]^+$.

*1-(3,4-Dichlorophenyl)-3-ethyl-2-methylisothiourea (**18c**).* Yield: 98%. Colorless oil. ^{1}H NMR ($CDCl_3$): δ 7.29 (d, J = 8.49 Hz, 1H), 7.00 (d, J = 2.40 Hz, 1H), 6.73 (dd, J = 8.49 Hz, J = 2.39 Hz, 1H), 4.45 (br, 1H), 3.63 (q, J = 6.56 Hz, 2H), 2.28 (s, 3H), 1.21 (t, J = 7.16 Hz, 3H). ^{13}C NMR ($CDCl_3$): δ 149.4, 132.3, 130.4, 125.6, 124.1, 122.0, 38.0, 14.7, 14.0. IR: 3115, 1605, 1519, and 1468 cm^{-1}. MS (EI): m/z 262.0 $[M]^+$.

*1-(Adamantan-1-yl)-3-(3,4-dichlorophenyl)-2-methylisothiourea (**18d**).* Yield: 99%. Mp 111−112 °C. White solid. ^{1}H NMR ($CDCl_3$): δ 7.29 (d, J = 8.49 Hz, 1H), 6.98 (d, J = 2.34 Hz, 1H), 6.74 (dd, J = 8.49 Hz, J = 2.34 Hz, 1H), 4.30 (s, 1H), 2.14 (s, 3H), 2.07 (m, 9H), 1.66 (m, 6H). ^{13}C NMR ($CDCl_3$): δ 149.3, 132.1, 130.1, 124.9, 123.8, 121.7, 53.9, 41.7, 36.3, 29.5, 15.0. IR (neat): 2910, 1583, 1528, and 1473 cm^{-1}. MS (EI): m/z 368.0 $[M]^+$.

*General Procedure for the Preparation of Compounds **19a**−**b**.* 3,4-Dichlorophenyl isothiocyanate (**16**) (4.0 g, 19.6 mmol) in 10.0 mL of CH_2Cl_2 was added dropwise to cyclic amine (29.4 mmol) in CH_2Cl_2 (20 mL). The mixture was stirred at room temperature until the starting material isothiocyanate **16** was consumed. The crude product was suspended in Et_2O (50 mL), and the solid product was collected and dried. The compound was pure enough for the next step synthesis without further purification.

*Pyrrolidine-1-carbothioic Acid (3,4-Dichlorophenyl)amide (**19a**).* Yield: 92%. Mp 187−188 °C. White solid. ^{1}H NMR (DMSO-d_6): δ 9.12 (s, 1H), 7.78 (d, J = 2.0 Hz, 1H), 7.48 (m, 2H), 3.64 (m, 4H), 1.82 (m, 4H). ^{13}C NMR (DMSO-d_6): δ 177.4, 141.3, 130.2, 129.9, 126.8, 126.1, 125.5, 65.3, 44.9, 24.1, 15.6. IR (neat): 3400, 2917, 1579, 1531, 1422, and 1306 cm^{-1}. MS (EI): m/z 274.0 $[M]^+$.

*Piperidine-1-carbothioic Acid (3,4-Dichlorophenyl)amide (**19b**).* Yield 98%. Mp 199−200 °C. White solid. ^{1}H NMR (DMSO-d_6): δ 9.31 (s, 1H), 7.59 (d, J = 2.19 Hz, 1H), 7.47 (d, J = 8.69 Hz, 1H), 7.29 (dd, J = 8.69 Hz, J = 2.19 Hz, 1H), 3.85 (m, 4H), 1.56 (m, 6H). ^{13}C NMR (DMSO-d_6): δ 180.6, 142.0, 130.3, 130.0, 126.4, 125.9, 125.1, 49.8, 25.9, 24.2. IR (neat): 3400, 2917, 1579, 1531, 1422, and 1306 cm^{-1}. MS (EI): m/z 288.8 $[M]^+$.

*General Procedure for the Preparation of **20a**,**b**.* Methyl iodide (3.9 g, 27.30 mmol) was added dropwise to thiourea **19** (18.2 mmol) and acetone (50 mL) solution. The mixture was stirred at 60 °C for 4 h, and the solvent was evaporated to dryness. The product was suspended in Et_2O, collected, and washed with fresh Et_2O. The white solid was dissolved in 50 mL of H_2O and THF (1:1 v/v) mixed solvent. The solution was basified with 1.0 M K_2CO_3 solution to pH 10 and extracted 3 times with 50 mL of CH_2Cl_2. The extracts were combined, dried over Na_2SO_4, and evaporated to dryness to give the product **20** in over 96% yield and was used without further purification.

*N-(3,4-Dichlorophenyl)pyrrolidine-1-carboximidothioic Acid Methyl Ester (**20a**).* The title compound was prepared from **19a** and CH_3I in 90% yield as a colorless oil. ^{1}H NMR ($CDCl_3$): δ 7.27 (d, J = 8.56 Hz, 1H), 7.05 (d, J = 2.44 Hz, 1H), 6.79 (dd, J = 8.56 Hz, J = 2.44 Hz, 1H), 3.52 (m, 4H), 2.05 (s, 3H), 1.92 (m, 4H). ^{13}C NMR ($CDCl_3$): δ 154.2, 149.8, 132.0, 130.0, 123.8, 123.2, 121.4, 49.3, 25.3, 14.0. IR (neat): 3015, 1585, 1458, and 1387 cm^{-1}. MS (EI): m/z 288.0 $[M]^+$.

*N-(3,4-Dichlorophenyl)piperidine-1-carboximidothioic Acid Methyl Ester (**20b**).* Compound **20b** was prepared from **19b** and CH_3I in 93% yield as a colorless oil. ^{1}H NMR (DMSO-d_6): δ 7.70 (m, 2H), 7.31 (m, 1H), 3.75 (m, 4H), 2.42 (s, 3H), 1.68 (m, 6H). ^{13}C NMR (DMSO-d_6): δ 168.7, 139.5, 132.0, 131.6, 128.8, 125.4, 123.9, 53.1, 25.8, 23.3, 17.0. IR (neat): 3015, 1583, 1448, and 1238 cm^{-1}. MS (EI): m/z 302.0 $[M]^+$.

*General Procedure for the Preparation of Compounds **21a**,**b**.* A reaction mixture consisting of 2-aminoimidazolin-4-one (**15b**, 1.0 g, 7.08 mmol), 2-methylisourea **20** (10.6 mmol), K_2CO_3 (1.0 g, 7.08 mmol), and 1-propanol (50 mL) was refluxed for 12 h and, after cooling to room temperature, was filtered to remove the K_2CO_3. The filtrate was evaporated to dryness under reduced pressure. The crude product was purified with a silica gel column using CH_2Cl_2/MeOH (10:1 v/v) as eluent. The product was further purified by recrystallization from ethyl acetate/MeOH mixed solvent.

*N-(3,4-Dichlorophenyl)-N′-(1-isopropyl-4-oxo-4,5-dihydro-1H-imidazol-2-yl)pyrrolidine-1-carboxamidine (**21a**).* The title compound was prepared from **20a** and **15b** in 50% yield as white crystals. Mp 177−178 °C. ^{1}H NMR ($CDCl_3$): δ 7.33 (d, J = 8.57 Hz, 1H), 7.14 (d, J = 2.40 Hz, 1H), 6.89 (dd, J = 8.57 Hz, J = 2.40 Hz, 1H), 4.57 (q, J = 6.70 Hz, 1H), 3.72 (s, 2H), 3.34 (m, 4H), 1.85 (m, 4H), 1.21 (s, 3H), 1.19 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 185.2, 171.0, 156.8, 138.6, 132.8, 130.5, 128.1, 124.9, 122.7, 49.5, 47.1, 44.0, 25.2, 20.5. IR (neat): 3000, 1681, 1555, and 1425 cm^{-1}. MS (ESI): m/z 382.01 $[M + 1]^+$. Anal. ($C_{17}H_{21}Cl_2N_5O$) C, H, N, Cl.

*N-(3,4-Dichlorophenyl)-N′-(1-isopropyl-4-oxo-4,5-dihydro-1H-imidazol-2-yl)piperidine-1-carboxamidine (**21b**).* The title compound was prepared from **20b** and **15b** in 48% yield as white crystals. Mp 195−197 °C. ^{1}H NMR ($CDCl_3$): δ 7.33 (d, J = 8.65 Hz, 1H), 7.14 (d, J = 2.55 Hz, 1H), 6.91 (dd, J = 8.65 Hz, J = 2.55 Hz, 1H), 4.57 (q, J = 6.74 Hz, 1H), 3.73 (s, 2H), 3.41 (m, 4H), 1.60 (m, 6H), 1.22 (s, 3H), 1.19 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 185.1, 171.6, 157.8, 139.5, 133.1, 130.8, 127.3, 122.4, 120.3, 48.3, 47.1, 44.2, 25.5, 24.2, 20.6. IR (neat): 1711, 1632, 1502, and 1438 cm^{-1}. MS (ESI): m/z 396.1 $[M + 1]^+$. Anal. ($C_{18}H_{23}Cl_2N_5O$) C, H, N, Cl.

*Synthesis of 2-Amino-1-(3,4-dichlorophenyl)-1,5-dihydroimidazol-4-one (**24**).* 3,4-Dichlorophenylcyanamide (**22**, 3.1 g, 16.5 mmol) in 20 mL of EtOH was added sodium metal (0.38 g, 16.5 mmol). The mixture was stirred at room temperature until sodium was consumed. The solvent was evaporated to dryness, and the residue was dissolved in 50 mL of CH_3CN. Chloroacetamide (1.7 g, 18.1 mmol) was added to the solution, and the mixture was refluxed for 5 h. When the mixture was cooled, the white solid precipitates were collected, washed successively with H_2O and acetone, and dried over Na_2SO_4 to give 2.7 g (67%) of compound **24**. Mp 285−287 °C. ^{1}H NMR (DMSO-d_6): δ 7.66 (m, 4H), 7.35 (m, 1H), 4.21(s, 2H). ^{13}C NMR (DMSO-d_6): δ 184.1, 169.5, 137.9, 132.1, 131.6, 127.0, 126.7, 125.0, 56.9. IR (neat): 3400, 1570, 1531, and 1420 cm^{-1}. MS (ESI): m/z 244.1 $[M + 1]^+$.

*General Procedure for the Preparation of **25a**−**c**.* 2-Amino-1-(3,4-dichlorophenyl)-1,5-dihydroimidazol-4-one (**24**, 4.0 g, 16.39 mmol), K_2CO_3 (1.3 g, 17.6 mmol), and 2-methylisothiourea **18** (25 mmol) were suspended in 1-propanol (25 mL) and DMF (5 mL) mixed solvent and was refluxed at 110 °C for 48 h. The solvent was removed under the reduced pressure. The crude product was dissolved in 20 mL of CH_2Cl_2 and washed with 15 mL of water. The organic layer was dried over Na_2SO_4 and evaporated to dryness. The product was purified with a silica gel column, followed by recrystallization from MeOH.

*N-(3,4-Dichlorophenyl)-N′-[1-(3,4-dichlorophenyl)-4-oxo-4,5-dihydro-1H-imidazol-2-yl]-N″-isopropylguanidine (**25a**).* The title compound was prepared from **24** and **18a** in 70% yield as white crystals. Mp 208−210 °C. ^{1}H NMR $CDCl_3$): δ 11.98 (br s, 1H), 8.19 (br s, 1H), 7.35−7.51 (m, 3H), 7.08−7.22 (m, 3H), 4.86 (s, 1H), 4.27 (m, 1H), 4.18 (s, 2H), 1.26 (s, 3H), 1.24 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 182.6, 170.7, 156.2, 137.8, 135.0, 134.8, 132.4, 131.7, 127.8, 127.2, 125.0, 123.1, 119.2, 52.2, 44.3, 22.8. IR (neat): 1700, 1574, 1470, 1384. MS (EI): m/z 473.0 $[M]^+$. Anal. ($C_{19}H_{17}Cl_4N_5O$) C, H, N, Cl.

*N-tert-Butyl-N′-(3,4-dichlorophenyl)-N″-[1-(3,4-dichlorophenyl)-4-oxo-4,5-dihydro-1H-imidazol-2-yl]guanidine (**25b**).* The title compound was prepared from **24** and **18b** in 65% yield as white crystals. Mp 167−168 °C. ^{1}H NMR ($CDCl_3$): δ 12.01 (br s, 1H), 7.88 (s, 1H),

Table 1. Causal Prophylactic Activity, Metabolic Stability, and in Vitro Potency[a]

		metab stability $t_{1/2}$ (min)			*P. falciparum* IC_{50} (μg/mL)			
compd	oral dose[b] (mg/kg)	human	mouse	EE *P. berghei*	D6	TM	W2	cLogP
8a	160	>60	>60	inactive	3.42	3.31	2.57	1.35
8b	160	>60	40	inactive	3.42	3.31	2.57	2.23
	40			DP: 6d (1/5)				
8c	160	>60	>60	CP: (2/5)	2.75	>5.00	1.01	2.58
				DP: 2-3d (1/5)				
	320			CP: (5/5)				
8d	160	<10	<10	CP: (4/5)	1.26	2.54	1.72	
				DP: 1d (1/5)				
	40			DP: 2-3d (2/5)				
8e	160	12.77	15.76	CP: (5/5)	1.60	>5	0.74	4.35
8f	160	<10	<10	CP: (5/5)	2.90	>5.0	>5.0	4.70
8g	320	34.3	>60	CP: (2/5)	2.86		2.80	3.66
				DP: 2-3d (2/5)				
	160			DP: 2-11d (2/5)				
				inactive: (3/5)				
8h	160	>60	>60	CP: (3/5)	3.19	>1.28	4.90	3.90
				DP: 3d (1/5)				
	40			inactive				
8i	320	14	5	CP: (4/5)	0.06	0.16	0.09	4.61
				DP: 3d (1/5)				
	160			DP: 2-5d (4/5)				
				inactive: (1/5)				
8j	320	53	17	CP: (5/5)	1.65	>5.0	0.37	3.94
	160			CP: (4/5)				
				DP: 1d (1/5)				
14	160	>60	>60	inactive	>12	>12	>12	5.83
8k	160	39.72	1.72	inactive	3.0	5.2	1.49	4.29
8l		>60	>60		3.38	3.01	2.27	3.59
8m	160	20.90	22.33	inactive	1.55	2.60	2.51	5.94
8n	320	>60	49.82	CP: (4/5)	>5.0	>5.0	>5.0	3.06
	160			CP: (1/5)				
				DP: 2d (4/5)				
9a	320	53.74	13.51		1.1	2.19	0.70	1.4
9b	320	>60	>60		0.56	0.90	0.63	3.52
9c	320	>60	>60	inactive	0.15		0.25	2.83
9d	320	40.45	13.50	CP: (4/5)	4.65		4.87	3.07
				DP: 2d (1/5)				
	160			CP: (3/5)				
				DP: 2-4d (2/5)				
9e	320	59.2	>60	CP: (3/5)	0.78		1.55	3.78
				DP: 2d (2/5)				
21a	160	>60	>60	CP: (1/5)	>5.0	>5.0	2.2	2.76
				DP: 2d (4/5)				
21b	160	28	18	inactive	>5.0		1.98	3.32
25a	160	39.21	27.25	inactive	1.66	2.83	2.86	5.87
	320			DP: 1-2d (4/5)				
				inactive: (1/5)				
25b	160	>60	>60	inactive	2.45		2.58	6.22
25c	160	>60	>60	inactive	2.6	>5.0	>5.0	5.53

[a] TM = TM91C235; EE *P. berghei* = sporozoites infected mouse model; DP = delayed in patency; CP = causal prophylaxis. [b] The dose unit is (mg/kg)/day, and mice were treated for 3 consecutive days on days −1, 0, 1.

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Table 2. Causal Prophylactive Activity of Deoxo-IZ Analogues[a]

					results	
drug	dose (mg/kg/day)	days treated	vehicle	route	first day patency	delayed patency (day)
vehicle	N/A	−1, 0, 1	HECT	po	8	valid control
					8	valid control
PQ	1.78	−1, 0, 1	MC	po	13	5
					13	5
TQ	0.316	−1, 0, 1	MC	po	11	3
					10	2
Malarone	14	−1, 0, 1	HECT	po	11	3
					12	4
8e	50	−1, 0, 1	HECT	po	12	4
					10	2
8j	50	−1, 0, 1	HECT	po	13	5
					12	4
8h	50	−1, 0, 1	HECT	po	8	0
					8	0

[a] PQ = primaquine; TQ = tafenoquine; DP = delayed parasitemia patency; Malarone = atovaquone (10 mg/kg) + proguanil (4 mg/kg).

7.51−7.48 (d, J = 8.50 Hz, 1H), 7.42−7.35 (m, 2H), 7.19 (d, J = 2.41 Hz, 1H), 7.09−7.06 (dd, J = 8.50 Hz, J = 2.41 Hz, 1H), 4.96 (br s, 1H), 4.20 (s, 2H), 1.37 (s, 9H). ^{13}C NMR ($CDCl_3$): δ 183.0, 170.8, 156.4, 137.6, 135.4, 134.0, 132.5, 131.6, 131.3, 130.2, 128.4, 127.6, 125.3, 124.8, 121.5, 53.1, 53.0, 29.5. IR (neat): 1698, 1567, 1470, and 1390 cm^{-1}. MS (ESI): m/z 487.93 $[M + 1]^+$. Anal. ($C_{20}H_{19}Cl_4N_5O$) C, H, N, Cl.

N-(3,4-Dichlorophenyl)-N′-[1-(3,4-dichlorophenyl)-4-oxo-4,5-dihydro-1H-imidazol-2-yl]-N″-ethylguanidine (***25c***). The title compound was prepared from **24** and **18c** in 66% yield as white crystals. Mp 229−231 °C ^{1}H NMR (DMSO-d_6): δ 10.30 (s, 1H), 9.21 (s, 1H), 7.87 (d, J = 8.50 Hz, 1H), 7.58−7.64 (m, 3H), 7.29−7.32 (m, 3H), 4.21 (s, 2H), 3.36 (q, J = 6.70 Hz, 2H), 1.14 (t, J = 6.65 Hz, 3H). ^{13}C NMR (DMSO-d_6): δ 182.0, 170.1, 156.7, 138.4, 137.9, 130.7, 130.2, 139.7, 127.3, 125.5, 124.6, 122.0, 120.0, 52.0, 36.4, 14.3. IR (neat): 1699, 1558, 1474, and 1395 cm^{-1}. MS (ESI): m/z 460.0 $[M + 1]^+$. Anal. ($C_{18}H_{15}Cl_4N_5O$) C, H, N, Cl.

B. Biological Studies. *(i) Assessment of Metabolic Stability.* The metabolic stability assay 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. The parent drug was quantified using external calibration and plots of parent drug response vs amount. The results are shown in Table 1.

(ii) In Vitro Antimalarial Studies. The in vitro assays were conducted by using a modification of the semiautomated microdilution techniques of Desjardins et al. and Chulay et al.[27,28] Three *P. falciparum* malaria parasite clones, from CDC Indochina III (W-2), CDC Sierra Leone I (D-6), and Southeast Asia Isolates (TM91C235), were utilized in susceptibility testing. They were derived by direct visualization and micromanipulation from patient isolates.[29] The results are shown in Table 1.

(iii) Assessment of Causal Prophylactic Activity in Mice. New compounds were assessed for their causal prophylactic activity in exoerythrocytic (EE) mouse model using sporozoites of *P. berghei*. The procedures have been previously described.[18−20] Briefly, each compound was ground with a mortar and pestle and suspended in hydroxyethylcellulose and Tween 80 for compounds to be administered po. 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 3 consecutive days to mice 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 and 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 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.

(iv) Assessment of Causal Prophylactic/Radical Curative Activities in Rhesus Monkeys. The causal prophylactic and radical curative antimalarial activity of the new derivatives **8e**, **8h**, and **8j** were assessed in *P. cynomolgi* sporozoites challenged Rhesus monkey model. Detailed procedures of sporozoite harvest and drug tests are described in the previous reports.[16−20] The results are shown in Table 2. Assessment of radical curative activity of the test compounds was 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) orally for 7 consecutive days and the test compounds by im dosing for 3 consecutive days after the parasitemia level reached 5000 parasites/mm^3. Chloroquine at 10 (mg/kg)/day × 7 days 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, 3 times per week for 4 weeks, and then 2 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 showing no relapse after 100 days are considered radically cured. Relapses of the control monkeys are treated with chloroquine once daily for 7 days and observed for second relapse. Relapse in experimental animals and the second relapse of the control monkeys are treated with the standard 7-day oral CQ and PQ (1.78 mg of base/kg). After standard treatment, blood smears were monitored daily for 4 consecutive days and 2 times weekly for 2 weeks. The results are shown in Table 3.

RESULTS AND DISCUSSION

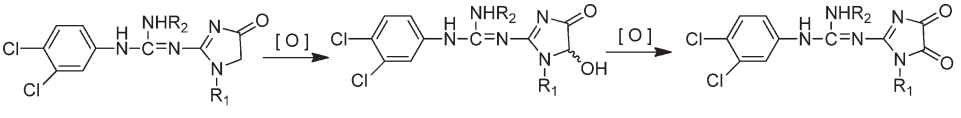
The test results of metabolic stability, 50% inhibitory concentration (IC_{50}) in three clones of *P. falciparum* (D-6, W-2, and TM91C235), and antimalarial activity in *P. berghei* sporozoites infected mice of the new compounds are summarized in Table 1. Calculated cLogP values were also included in the table to facilitate the examination of the relationship between lipophylicity and antimalarial activity of the new compounds. The results indicated that the cLogP values of the new compounds with good in vitro and in vivo activity are well within 3−4.5, a value shared by the majority of the exiting antimalarial drugs. As was observed with imidazolinedione (IZ) derivatives (**1**−**6**), the deoxo-IZ analogues (**8**, **9**) prepared in this study exhibited only weak in vitro activity against blood stage malaria, *P. falciparum*, except **8j**, **8h**, **9b**, and **9c**, which showed moderate activity with IC_{50} less than 1 μg/mL.

The results also indicated that the deoxo-IZ compounds (**8**, **9**) are in general metabolically unstable, especially analogues with good prophylactic activity, such as **8d**, **8e**, **8f**, **8i**, and **8j**. To identify the active metabolites, extensive metabolic studies of the

Table 3. Radical Curative Activity of Deoxo-IZ Analogues

	drug 2			
drug 1		dose ((mg/kg)/day)	route	no. days delayed in relapse after treatment
chloroquine (10 mg/kg/day) po for 7 days	none			11
				11
	8e	30 × 3 days	im	radical cure
				radical cure
		50 × 7 days	po	28
				radical cure
	8j	30 × 3 days	im	42
				38
	8h	30 × 3 days	im	16
				15
	PQ	1.78 × 7 days	po	radical cure

Scheme 4. Major Metabolic Pathway of Imidazolin-4-one Derivatives



most active compounds **8e** and **8f** in mice were conducted. Preliminary results indicated that these compounds were metabolized extensively to yield a host of metabolites in mice. The major metabolites,[31] however, were identified as the corresponding hydroxyimidazolinones (II) and imidazolinediones (III) as shown in Scheme 4. For example, compounds **8e** and **8j** were metabolized to the corresponding IZ derivatives **8e′** and **8j′**, respectively. The LC/MS results of compounds **8e′** and **8j′** are identical to the results of the known compound **3** and the *N*,*N*′-diisopropyl-IZ analogue,[32] respectively, in LC retention time and mass spectrum. Since deoxo-IZ compounds **8e** and **8j** are metabolically vulnerable and their major metabolites (**8e′** and **8j′**) are potent antimalarials, most likely the new class of deoxo-IZ (I) may act as prodrugs of the IZ compounds (III) as shown in Scheme 4.

The SAR of the new compounds indicated that isopropyl is a preferred substituent at R_1 and R_2 of deoxo compounds **8** and **9** over the other groups. Among the nine compounds (**8b**, **8e**, **8g**, **8h**, **8i**, **8j**, **8k**, **8l**, and **8m**) with R_1 being isopropyl, six (**8e**, **8g**, **8h**, **8i**, **8j**, and **8l**) showed curative activity in the prophylactic test in mice. Compound **8j**, with both R_1 and R_2 being the isopropyl group, showed the best activity among the compounds tested, with 5/5 and 4/5 mice protected at doses of 320 and 160 mg/kg × 3, respectively. Besides the isopropyl group, *t*-Boc also appears to be a favorable substituent at R_2, as **8d**, **8e**, and **8f** all provided good protection to the infected mice at 160 mg/kg × 3 dosing.

On the basis of promising efficacy results in the mouse model, compounds **8e**, **8h**, and **8j** were selected for further testing in Rhesus monkeys infected with sporozoites of *P. cynomolgi* to assess their casual prophylactic and radical curative activities. Three clinical drugs, PQ, TQ, and Malarone, were also tested in the same experiment to determine their benchmark dosage as prophylactic drugs and to serve as positive control of the experiment. The primaquine dose of 1.78 mg/kg × 3 was used, as it cured 100% of the relapsed monkeys when in combination use with 10 mg/kg chloroquine for 7 days by oral route. The tafenoquine dose of 0.316 mg/kg was based on the report of Puri.[30] The Malarone dose (14 mg/kg) was derived from the recommended human dose. The results showed that both **8e** and **8j** showed comparable activity as that of PQ, TQ, and Malarone and delayed patency of the treated monkeys 2−5 days longer than the untreated control by oral administration (Table 2). As was reported earlier, the IZ class of compounds (**1**, **3**, and **5**) showed good casual prophylactic activity in Rhesus only by im but not by oral dosing. Although 2−3 days of delay in patency is far from impressive, this is the first time significant oral activity of this class of compounds was observed in Rhesus monkeys. Furthermore, the most commonly used clinical drugs, PQ, TQ and Malarone, also did not show much activity in the same test, suggesting the need to amend the current protocol used to assess the casual prophylactic activity in Rhesus. Compound **8h** is a carboxamide derivative that exhibited good casual prophylactic activity in mice, but no activity was observed in the same Rhesus test by im at 30 mg/kg × 3. The result further confirmed our previous observations that carboxamide-IZ derivatives, such as **5** and **6**, are generally less active than the corresponding *N*-alkyl or *N*-carbamate derivatives in the Rhesus test.[17,18,32]

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Monkeys failed in the causal tests were used for assessment of radical curative activity of **8e**, **8h**, and **8j** according to the method described above. The results are shown in Table 3. Compound **8e** at 30 mg/kg × 3 by im, combined with 10 mg/kg of CQ by oral, showed complete cure of both treated monkeys. No cure was observed for monkeys treated with **8j** and **8h**, but the relapse time was delayed from 11 days for untreated control to 40 days and 15.5 days for monkeys treated with **8j** and **8h**, respectively. Compound **8e**, the most active among the three tested, was further evaluated for oral radical curative activity at a dose of 50 (mg/kg)/day for 7 consecutive days. One of the treated monkeys was cured and the other was protected for 28 days. PQ at 1.78 mg/kg/day in combination with 10 mg/kg of CQ for 7 consecutive days by oral administration results in a complete radical cure.

The Rhesus PK data revealed that $t_{1/2}$ of compound **3** is much longer when the drug was administered by im (~70.5 h) than by oral (8.9 h) dosing, likely because of the depot effect of the drug at the injection sites.[31] Although absorption factors remain unknown via im route, a compound with a half-life of 70.5 h would take roughly 14 days to clear 97% of the absorbed dose. Our previous studies also indicated that compound **3** showed good protective activity in Rhesus by im, but not by oral dosing.[17−19] Further PK study showed the poor oral activity of **3** was not due to poor oral absorption. In fact, compound **3** was well absorbed by oral route; peak absorption of a 60 mg/kg dose given orally was nearly 10-fold higher than 30 mg/kg dose by im route. The Rhesus PK and the efficacy data suggested that long plasma half-life of the drug is critical for its activity against liver stage malaria. Furthermore, analysis of the relative presence of metabolites indicated that compound **3** is the major species, comprising 86% and 96% of measurable analytes at 24 h via the oral and im routes, respectively. A small amount (4−10%) of the metabolites is the original lead compound **1** and a very small amount (<0.5%) is metabolized to *s*-triazenes (**7**) at 24 h, suggesting that the parent compound **3** is the active species.[31] As the major metabolite of the deoxo-IZ (I) is its corresponding IZ derivative III (Scheme 4) and the latter is highly active in mouse and monkey models, the deoxo-IZ (I) analogues prepared in this study may act as prodrugs of the corresponding IZ derivative (III).

CONCLUSION

New 2-guanidino-4-oxoimidazoline derivatives (deoxo-IZ) were prepared and showed potent antimalarial activities in rodent and Rhesus models. Compound **8e** is the first non-8-aminoqinoline antimalarials that demonstrated radical curative activity in non-human primate by oral route and showed causal prophylactic activity comparable to that of the commonly used clinical drugs in the Rhesus model. The metabolic stability and metabolites profile indicated that the new deoxo-IZ derivatives (**8**) may act as prodrugs of the corresponding IZ derivatives.

ASSOCIATED CONTENT

Supporting Information. Elemental analysis data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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ABBREVIATIONS USED

PQ, primaquine; TQ, tafenoquine; DP, delayed parasitemia patency; Malarone, atovaquone (10 mg/kg) + proguanil (4 mg/kg); IZ, guanidylimidazolinedione; G6PD, glucose 6-phosphate dehydrogenase

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After this paper was published online May 31, 2011, some minor corrections were made to Table 1. The revised version was published June 2, 2011.