Antimalarial Activities of New Guanidylimidazole and Guanidylimidazoline Derivatives

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Ⓢ *Supporting Information*

ABSTRACT: A series of new guanidylimidazole derivatives was prepared and evaluated in mice and Rhesus monkeys infected with malarial sporozoites. The majority of the new compounds showed poor metabolic stability and weak in vitro activities in three clones of *Plasmodium falciparum.* Compounds **8a**, **8h**, **9a**, **16a**, and **16e** cured the mice infected with sporozoites of *P. berghei* at 160 and 320 mg/kg/day × 3 po. Compounds **8a** showed better causal prophylactic activity than primaquine, tafenoquine, and Malarone in the Rhesus test. In the radical curative test, **8a** cured one monkey and delayed relapse of another for 74 days at 30 mg/kg/day × 7 by im. By oral dosing, **8a** delayed relapse 81 days for one and 32 days for other vs 11−12 days for control monkeys treated with 10 mg/kg of chloroquine by po alone. Compound **8h**, which showed superior activity to **8a** in mouse test, delayed the relapse of treated monkeys for 21−26 days at 30 mg/kg/day × 7 by oral.

R_1 = Alkyl
R_2, R_3 = H. alkyl, or -COR, wherein R = alkyl or alkoxyl

INTRODUCTION

Multiple drug resistance and lack of safe drugs to prevent or radically cure the malaria diseases continue to pose challenges to the containment of this deadly disease which have threatened the life of millions of people in the developing world.[1−5] Widespread drug resistance to chloroquine, the first line antimalarial drug, was reported in Southeast Asia and South America countries.[6−10] Central nervous system (CNS) toxicity of mefloquine[11] and hemolytic side effects of primaquine and tafenoquine in glucose-6-phosphate dehydrogenase (G6PD) deficient patients[7,8] have compromised the clinical value of these otherwise highly effective malaria therapeutic drugs. With the exception of quinolone esters, only the 8-aminoquinoline drugs such as primaquine (PQ) or tafenoquine (TQ) have activity against the liver stages of *Plasmodium vivax* and *Plasmodium falciparum* malarias.[12−15] Therefore, there is an eminent need for new and safe malaria drugs to replace PQ and TQ to treat and protect the tourists traveling in the endemic areas of the world.

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. Recently, a series of new 2-guanidylimidazolidinedione (IZ) derivatives (**1** and **2**, Figure 1) was demonstrated in our laboratory to possess causal prophylactic antimalarial activity in Rhesus monkeys infected with *Plasmodium cynomolgi* sporozoites.[16−21] Carbamate **3**, the most active compound of this class, protects 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 the Thompson mouse test against *Plasmodium berghei*, a blood stage rodent malaria.[16−20] 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 less likely to develop drug resistance. However, compound **3** showed poor activity by oral administration, delaying the patency (the first day that parasites can be detected in the blood smears after infection) of treated monkeys for only 3 days vs untreated control. Oral efficacy is an essential consideration in the search of prophylactic/radical curative antimalarial drugs.

Lack of oral activity of carbamates **3** and **4** in Rhesus testing may be attributed to the hydrolysis of carbamate group in stomach acid, leading to generation of insoluble parent compounds **1** and **2**. To overcome the acid instability of carbamates, a series of acid stable carboxamide derivatives **5** and **6** were prepared and again found to be active only by im; no activity was found by oral dosing in Rhesus.[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. Both compounds **5** and **6** were converted to the same *s*-triazine derivatives as shown in Scheme 1. This transformation was also observed when both compounds were

incubated in microsomal preparations and in phosphate buffer solution, indicating that the conversion of carboxamides **5** and **6** to *s*-triazine **7** is a chemical rather than an enzymatic reaction.[20] Because both carboxamides showed causal prophylactic activity in Rhesus and the corresponding *s*-triazine are inactive, *s*-triazine was ruled out as the possible active metabolite of compounds **5** and **6**. As compound **5** is more stable and more efficacious in Rhesus than compound **6**, it was speculated that the parent compound possesses intrinsic antimalarial activity, not its *s*-triazine metabolite.

The synthesis of carbamates **3** and **4** and later the carboxamides **5** and **6** derivatives of imidazolidinediones **1** and **2** were based on the assumption that carboxamides are more stable chemically than carbamates. However, as shown in Scheme 1, although it is an amide, the imidazolidinedione ring is not as stable as one might think, especially when the 5-membered ring carries an electron withdrawing dichlorophenyl substituent (**2** and **4**). The incorporation of two electron withdrawing carboxy groups at 4- and 5-positions of the imidazolidine makes
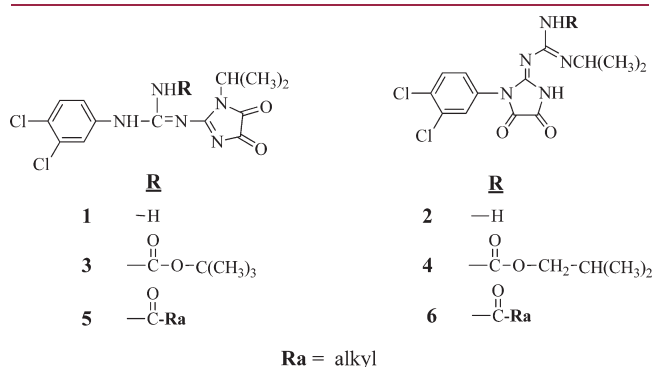
the 5-membered ring highly strained and susceptible to nucleophilic assaults. In this study, we report the synthesis and antimalarial activity of a series of chemically stable imidazole and imidazoline analogues. The chemically unstable imidazolidinedione ring of the IZ molecules (**1**−**4**) were replaced with a stable imidazole (**8a**−**m**) and/or imidazoline (**9a**−**e**) rings as shown in Figure 2.

■ CHEMISTRY

1. Synthesis of Imidazole Analogues (8a−m). Two approaches were used to prepare the new compounds **8a**−**m**. The first approach involved the coupling between N^1-substituted-N^2-(3,4-dichlorophenyl)-thiourea (**11a**−**c**) and 2-aminoimidazole (**14a**−**d**) to prepare compound **8a**−**f** under the catalysis of HgO (Scheme 2). The second approach employed the key intermediate, N^1-(3,4-dichlorophenyl)-N^2-(2-imidazolyl)-thiourea (**15a**−**d**), to react with appropriate substituted amines under the catalysis of HgCl$_2$ (Scheme 3). The difference between the two approaches is the sequence of incorporation of 2-aminoimidazole group into the final products. The products prepared by both methods are identical, but the latter approach gave better yields than the former.

The key intermediates, thiourea derivatives **11a**−**c**, were prepared in high yields by treatment of 3,4-dichlorophenyl-isothiocyanate (**10**) with substituted amines in CH$_2$Cl$_2$ at room temperature overnight. The other intermediates, 2-aminoimidazoles (**14a**−**d**), were prepared according to a reported procedure[22] by treatment of 2-bromo-1,1-dimethoxyethane (**12**) with appropriate amines to give 2-alkylamino-dimethoxyacetals (**13a**−**d**). The latter compounds were heated with cyanoamine in a mixture of 50% AcOH/conc HCl for an hour at 100 °C, followed by hydrolysis in conc KOH to give 2-aminoimidazoles (**14a**−**d**) with yields ranging from 47% to 70%. The coupling of

Figure 1. Guanidylimidazolidinedione derivatives.

Scheme 1. Mechanism of Chemical Transformation from Carboxamides 5 and 6 to *s*-Triazine 7
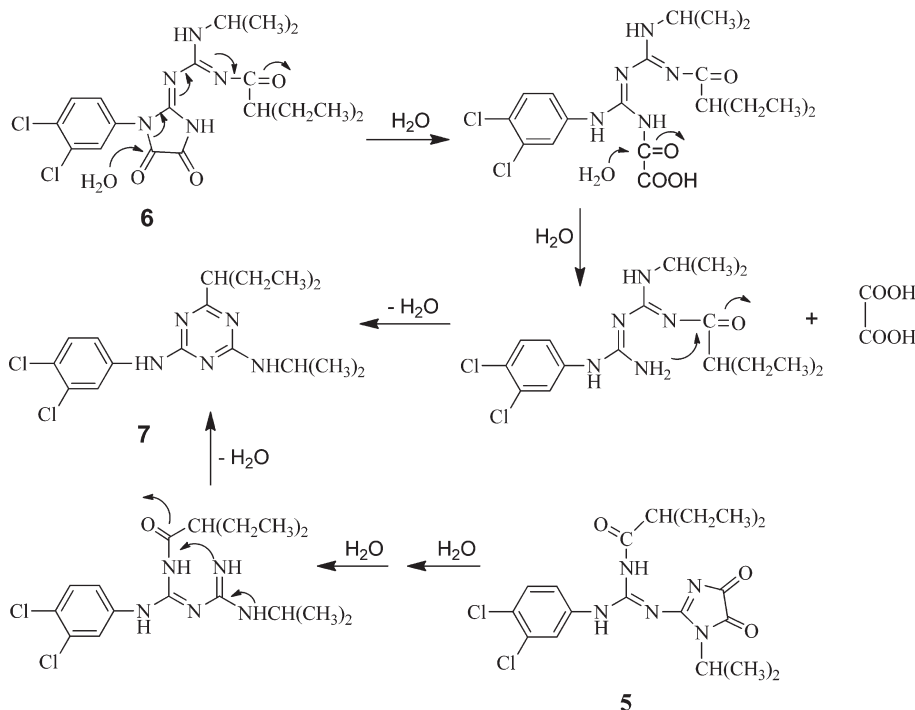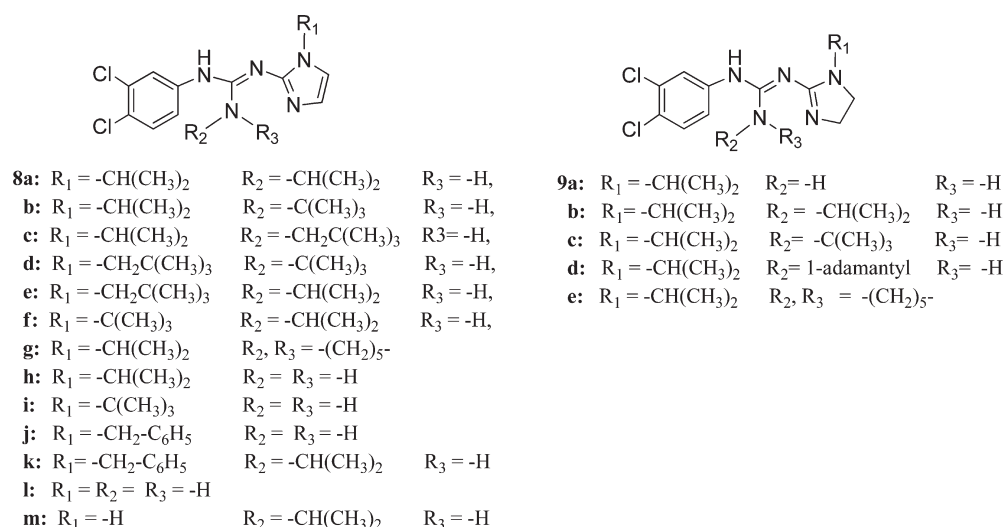
Figure 2. Imidazole **8a**—**m** and imidazoline **9a**—**e** Analogues.

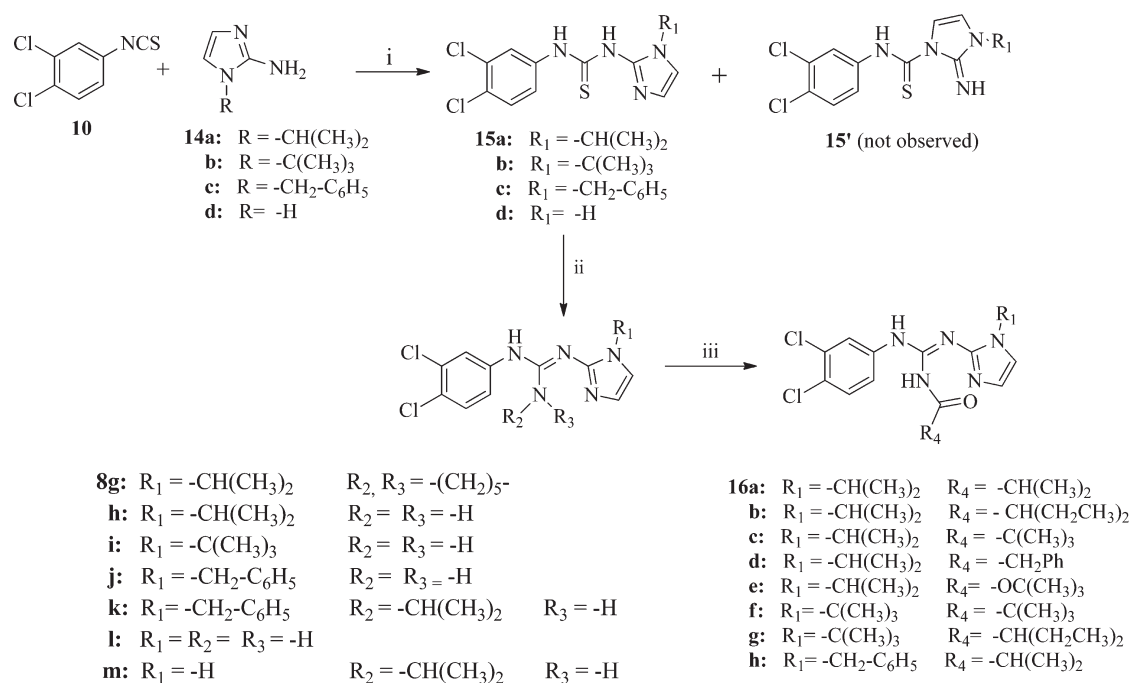**Scheme 2. Synthesis of Imidazole Analogues 8a—f**[a]
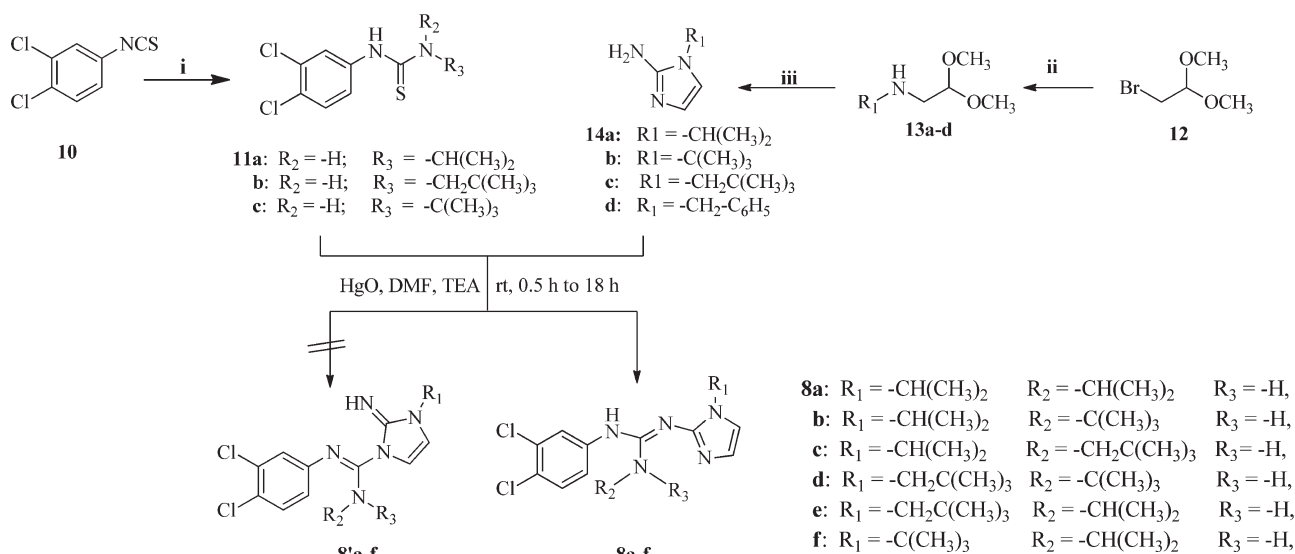
[a] Reagents and conditions: (i) substituted amines, CH_2Cl_2, rt, 18 h; (ii) substituted amines, sealed tube, 80—100 °C, neat, 18 h; (iii) (a) NH_2CN, HOAc (50% aq), HCl (10 M), 100 °C, 1 h; (b) KOH (50%).

thioureas (**11a**—**c**) and 2-aminoimidazoles (**14a**—**d**) was facilitated by mercuric oxide as catalyst to give the desired products **8a**—**f** in 30—54% yields. Without HgO as catalyst, the thioureas failed to react with the 2-aminoimidazoles. Theoretically, the coupling of **11a**—**c** with compounds **14a**—**d** should give, besides the desired products **8a**—**f**, other isomers **8′a**—**f**.[23] However, the reaction gave exclusively the desired products **8a**—**f**.
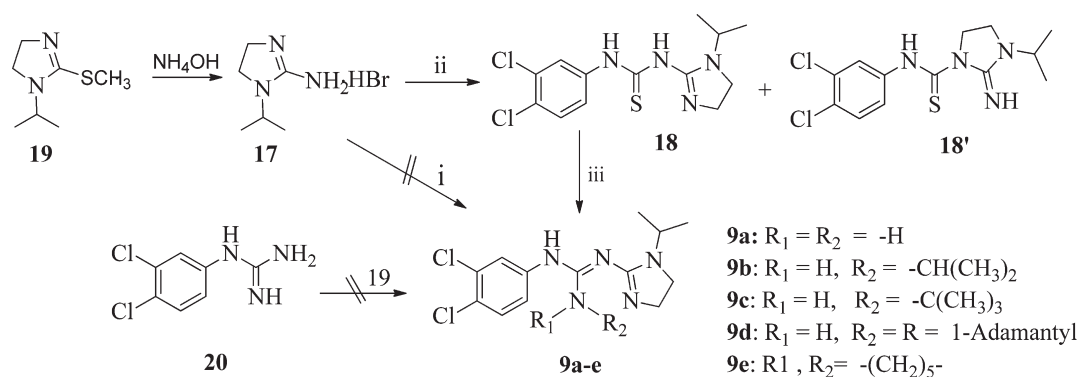
Because the NMR spectrum of both isomers **8a** and **8a′** are too close to be distinguished, the confirmation of the structure **8a** relied mainly on the single crystal X-ray crystallography technique. The result is as shown in Figure 3 (Supporting Information p S-1: X-ray crystallography of N-(3,4-dichlorophenyl)-N′-isopropyl-N″-(1-isopropyl-1H-imidazol-2-yl)-guanidine (**8a**)).

An alternative method to prepare compounds **8g**—**m** was accomplished by treating 3,4-dichlorophenylisothiocyanate (**10**)

with 2-aminoimidazoles **14a**—**d** to give compounds **15a**—**d** (Scheme 3). The structure of compound **15a** was confirmed by single-crystal X-ray diffraction data as shown in Figure 4 (Supporting Information p S-2: X-ray crystallography of 1-(3,4-dichlorophenyl)-3-(1-isopropyl-1H-imidazol-2-yl)-thiourea (**15a**)). Upon reaction with appropriate amines under the catalysis of $HgCl_2$, compounds **15a**—**d** gave the desired products **8a**—**c** and **8g**—**m**. Mercuric chloride facilitated the coupling reaction.[24] However, it can be replaced with a nontoxic dicyclohexylcarbodiimide (DCC), as exemplified by the synthesis of **8m**. The ^{1}H and ^{13}C NMR spectrum and the TLC R_f value of **8a** prepared by both methods are identical. It is to be noted that HgO cannot be used to substitute for $HgCl_2$ as catalyst in the coupling reaction between thioureas and amines as shown in Scheme 3. Conversely, $HgCl_2$ cannot be used to replace HgO as catalyst in a

Scheme 3. Synthesis of 8g−m and 16a−h[a]

8g: R_1 = -CH(CH$_3$)$_2$ R_2, R_3 = -(CH$_2$)$_5$-
h: R_1 = -CH(CH$_3$)$_2$ R_2 = R_3 = -H
i: R_1 = -C(CH$_3$)$_3$ R_2 = R_3 = -H
j: R_1 = -CH$_2$-C$_6$H$_5$ R_2 = R_3 = -H
k: R_1= -CH$_2$-C$_6$H$_5$ R_2 = -CH(CH$_3$)$_2$ R_3 = -H
l: R_1 = R_2 = R_3 = -H
m: R_1 = -H R_2 = -CH(CH$_3$)$_2$ R_3 = -H

16a: R_1 = -CH(CH$_3$)$_2$ R_4 = -CH(CH$_3$)$_2$
b: R_1 = -CH(CH$_3$)$_2$ R_4 = - CH(CH$_2$CH$_3$)$_2$
c: R_1 = -CH(CH$_3$)$_2$ R_4 = -C(CH$_3$)$_3$
d: R_1 = -CH(CH$_3$)$_2$ R_4 = -CH$_2$Ph
e: R_1 = -CH(CH$_3$)$_2$ R_4= -OC(CH$_3$)$_3$
f: R_1= -C(CH$_3$)$_3$ R_4 = -C(CH$_3$)$_3$
g: R_1= -C(CH$_3$)$_3$ R_4= -CH(CH$_2$CH$_3$)$_2$
h: R_1= -CH$_2$-C$_6$H$_5$ R_4 = -CH(CH$_3$)$_2$

[a] Reagents and conditions: (i) CH$_2$Cl$_2$, rt, 18 h; (ii) ammonia, or substituted amine, HgCl$_2$, DMF or CH$_3$CN, rt, 30 min to 18 h, or substituted amine, i-Pr$_2$NEt, DCC, CH$_3$CN, rt, 18 h; (iii) RCOCl, TEA, CHCl$_3$ (for **16e**, RCOOCOR was used).

Scheme 4. Synthesis of Imidazolidine Analogues 9a−e[a]

9a: R_1 = R_2 = -H
9b: R_1 = H, R_2 = -CH(CH$_3$)$_2$
9c: R_1 = H, R_2 = -C(CH$_3$)$_3$
9d: R_1 = H, R_2 = R = 1-Adamantyl
9e: R1 , R_2= -(CH$_2$)$_5$-

[a] Reagents and conditions: (i) 3,4-dichlorophenylcyanamide, EtOH, reflux, 24 h; (ii) 3,4-dichlorophenyl isothiocyanate, CH$_2$Cl$_2$, rt, 24 h; (iii) ammonia or substituted amines, HgCl$_2$, CH$_3$CN or DMF, rt, 10 min to 18 h.

coupling reaction between **11a−c** and 2-aminoimidazoles (**14a**) as shown in Scheme 2. A series of carboxamide and carbamate derivatives **16a−h** were synthesized from amine **8h−j** as shown in Scheme 3.

2. Synthesis of Imidazoline Analogues (9a−e). Approaches used for the synthesis of imidazoline analogues **9a−e** were as shown in Scheme 4. 3,4-Dichlorophenyl isothiocyanate **10** was reacted smoothly with 2-aminoimidazoline **17**, yielding two isomers **18** (27% yield) and **18′** (50% yield), which were separated via a column chromatography.[25] The structure of thiourea **18** was determined by single crystal X-ray crystallography as shown in Figure 5 (Supporting Information p S-3: X-ray crystallography of 1-(3,4-dichlorophenyl)-3-(1-isopropyl-4,5-dihydro-1H-imidazol-2-yl)-thiourea (**18**)). The conversion of thiourea **18** to the final products **9a−e** was carried out by using the same procedure for

the preparation of **8g−m**, with HgCl$_2$ as catalyst in CH$_3$CN or DMF (Scheme 3). Attempts were made to improve the yield of **18** and minimize the byproduct **18′** formation using various solvents, i.e., hexanes, CHCl$_3$, CH$_3$CN, DMF, THF, Et$_2$O, EtOH, or Et$_3$N but failed.

Initially, we envisioned that mercuric chloride, lead chloride, or mercuric oxide which facilitated the synthesis of imidazole derivatives (**8g−m**) could be used to assemble the imidazoline analogues (**9a−e**).[26] However, coupling reaction between **11a** and imidazoline **17** failed to give the desired compound **9b**. Likewise, heating of **17** with 3,4-dichlorophenylcyanamide in EtOH under reflux for 24 h also did not yield **9a**. Furthermore, treatment of 3,4-dichlorophenylguanidine **20** with 1-isopropyl-2-methylmercapto-imidazoline **19** did not provide the desired **9a**.

■ EXPERIMENTAL SECTION

Melting points were determined in open capillary tubes on an OptiMelt melting point apparatus (Standard Research Systems, USA) and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded using Bruker Avance-300 and Bruker Avance 600 spectrometers (Bruker Instrument, 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 or iodine chamber. 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 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 purity analysis and chromophore properties. The system consisted of an Agilent 1100 series LC-UV/vis system online with a ThermoFinnigan (now Thermo-Fisher Scientific, Waltham, MA) LCQ MS equipped with an electro spray ionization (ESI) source. Samples were analyzed using shallow CH3CN: 1% HCOOH/H2O gradients at low flow rate. The purity of the final products is ≥95%.

1. Synthesis of Compounds 11a−c. *Synthesis of 1-(3,4-Dichlorophenyl)-3-isopropyl-thiourea (11a)*[27]. 3,4-Dichlorophenyl-isocyanate (7.3 mL, 50.0 mmol) in CH2Cl2 (40.0 mL) was added dropwise with stirring to a solution of isopropylamine (8.6 mL, 0.10 mol) in CH2Cl2 (20.0 mL) at ambient temperature. After the addition of the amine was completed, the reaction mixture was further stirred at rt for 18 h. The solvent was removed under reduced pressure. The residue was suspended in diethyl ether and filtered. The crude product was washed with diethyl ether, followed by hexanes to give 11a as a white solid (12.8 g, 97% yield), mp 145.0−146.5 °C. ^{1}H NMR (CDCl3): δ 1.24 (d, $J = 6.5$ Hz, 6 H), 4.55 (m, 1 H), 5.75 (br s, 1 H), 7.08 (dd, $J = 2.4$ Hz, and 8.5 Hz, 1 H), 7.33 (d, $J = 2.4$ Hz, 1H), 7.50 (d, $J = 8.5$ Hz, 1 H), 7.74 (br s, 1 H). MS (ESI): m/z 263 [M + 1]$^+$.

Compounds 11b−c were prepared by the same procedure, except neopentylamine or *tert*-butylamine was used in replace of isopropylamine.

1-(3,4-Dichlorophenyl)-3-(2,2-dimethyl-propyl)-thiourea (11b). The title compound was prepared according to the same procedure of making 11a, using neopentylamine in place of isopropylamine, to give a white crystalline solid in 93% yield, mp 148.0−149.5 °C. ^{1}H NMR (CDCl3): δ 0.99 (s, 9 H), 3.53 (d, $J = 5.0$ Hz, 2 H), 6.06 (br s, 1 H), 7.15 (dd, $J = 2.2$ and 8.5 Hz, 1 H), 7.42 (d, $J = 2.2$ Hz, 1 H), 7.56 (d, $J = 8.5$ Hz, 1 H), 7.62 (br s, 1 H). ^{13}C NMR (CDCl3): δ 27.5, 32.0, 60.5, 124.0, 126.6, 131.6, 134.0, 135.9, 180.9. MS (EI): m/z 290 [M]$^+$.

1-tert-Butyl-3-(3,4-dichlorophenyl)-thiourea (11c). The title compound was prepared according to the same procedure of making 11a, using *t*-butylamine in place of isopropylamine, to give a brown crystalline solid in 92% yield, mp 168.4−169.2 °C. ^{1}H NMR (CDCl3): δ 1.54 (s, 9 H), 6.00 (br s, 1 H), 7.10 (d, $J = 8.4$ Hz, 1 H), 7.35 (s, 1 H), 7.49 (d, $J = 8.4$ Hz, 1 H). ^{13}C NMR (CDCl3): δ 29.0, 54.4, 123.9, 126.3, 130.3, 131.4, 133.6, 136.6, 179.0. MS (EI): m/z 276 [M]$^+$.

2. Synthesis of Compounds 13a−d. *N-(2,2-Dimethoxy)-ethyl-N-isopropyl-amine (13a)*[22]. A mixture of 2-bromo-1,1-dimethoxyethane (50.0 mL, 0.423 mol) and isopropylamine (150 mL, 1.74 mol) was heated in a sealed tube at 80 °C for 18 h. The crystals of isopropylamine HBr salt were filtered off. The filtrate was concentrated under reduced pressure. Water (50 mL) was added to the gummy residue, and the mixture was extracted with CH2Cl2 (150 mL × 2). The organic extracts were combined, dried over Na2SO4, and concentrated under reduced pressure

to give a light-brown liquid product 13a (60.0 g, 96% yield). ^{1}H NMR (CDCl3): δ 1.07 (d, $J = 6.3$ Hz, 6 H), 2.73 (d, $J = 5.6$ Hz, 2 H), 3.40 (s, 6 H), 4.48 (t, $J = 5.6$ Hz, 1 H). ^{13}C NMR (CDCl3): δ 22.7, 48.4, 53.7, 53.8, and 103.9. MS (EI): m/z 147 [M]$^+$. The product was pure enough for next reactions without further purification.

Compounds 13b−d were prepared by the same procedure, except *t*-butylamine, neopentylamine, and benzylamine were used, respectively, in place of isopropylamine.

tert-Butyl-(2,2-dimethoxy-ethyl)-amine (13b). The title compound was obtained by the same method of preparing compound 13a, except, *t*-butylamine was used in place of isopropylamine to give as a light-brown liquid in 83% yield. ^{1}H NMR (CDCl3): δ 1.18 (s, 9 H), 2.71 (d, $J = 5.58$ Hz, 2 H), 3.40 (s, 6 H), 4.47 (t, $J = 5.48$ Hz, 1 H). MS (ESI): m/z 162 [M + 1]$^+$.

(2,2-Dimethoxy-ethyl)-(2,2-dimethyl-propyl)-amine (13c). The title compound was obtained as a light-brown liquid in 87% yield from 2,2-dimethylpropylamine using the same procedure to prepare compound 13a. ^{1}H NMR (CDCl3): δ 0.90 (s, 9 H), 2.35 (s, 2 H), 2.72 (d, $J = 5.6$ Hz, 2 H), 3.36 (s, 6 H), 4.48 (t, $J = 5.6$ Hz, 1 H). MS (EI): m/z 175 [M]$^+$.

Benzyl-(2,2-dimethoxy-ethyl)-amine (13d). The title compound was prepared in 98% yield by the same method of preparing compound 13a, using benzylamine in place of isopropylamine. ^{1}H NMR (CDCl3): δ 2.75 (d, $J = 5.4$ Hz, 2 H), 3.37 (s, 6 H), 3.81 (s, 2 H), 4.49 (t, $J = 5.4$ Hz, 1 H), 7.23−7.33 (m, 5 H).

3. Synthesis of Compounds 14a−d. *Synthesis of 1-Isopropyl-1H-imidazol-2-ylamine (14a)*[23]. N-Isopropyl substituted aminoacetal 13a (6.2 g, 42 mmol) was added dropwise to a stirring solution of cyanamide (5.2 g, 0.124 mol) in aqueous acetic acid (50%, 40 mL) at ambient temperature. The reaction mixture was then heated at 100 °C for 1 h. The solvent was removed under reduced pressure. The residue was dissolved in concentrated HCl (10 N, 20 mL), and the mixture was heated at 100 °C for 15 min. The pH of the mixture was adjusted to ~11 by addition of conc aqueous KOH solution (50%). The mixture was then extracted with CH2Cl2 (50 mL × 3). The CH2Cl2 extracts were combined and dried over Na2SO4, and the solvent was removed under reduced pressure to give desired product 14a as light-brown oil which partially solidified on standing (2.5 g, 47% yield). ^{1}H NMR (CDCl3): δ 1.39 (d, $J = 6.5$ Hz, 6 H), 4.14 (m, 1 H), 6.60 (d, $J = 1.6$ Hz, 1 H), 6.66 (d, $J = 1.6$ Hz, 1 H). MS (EI) m/z 125 [M]$^+$.

Compound 14a was pure enough for next step reactions without further purification. Compounds 14b−d were prepared by the same procedure, except 13b−d was used, respectively, in place of 13a.

1-tert-Butyl-1H-imidazol-2-ylamine (14b). A brown crystalline solid prepared from 13b and cyanamide in 69% yield, mp 100.8−101.5 °C. ^{1}H NMR (CDCl3): δ 0.98 (s, 9 H), 6.50 (d, $J = 2.4$ Hz, 1 H), 6.62 (d, $J = 2.4$ Hz, 1 H). MS (EI): m/z 153 [M]$^+$.

1-(2,2-Dimethyl-propyl)-1H-imidazol-2-ylamine (14c). A light-brown liquid prepared from 13c and cyanamide in 70% yield. ^{1}H NMR (CDCl3): δ 1.59 (s, 9 H), 3.93 (br s, 2 H), 6.59 (d, $J = 2.4$ Hz, 1 H), 6.69 (d, $J = 2.4$ Hz, 1 H). MS (EI): m/z 139 [M]$^+$.

1-Benzyl-1H-imidazol-2-ylamine (14d). Brown oil prepared from 13d and cyanamide in 82% yield. ^{1}H NMR (CDCl3): δ 3.81 (br s, 2 H), 4.93 (s, 2 H), 6.60 (s, 1 H), 6.68 (s, 1 H), 7.13−7.70 (m, 5 H). MS (EI): m/z 173 [M]$^+$.

4. Synthesis of Compounds 15a−d. *Synthesis of 1-(3,4-Dichlorophenyl)-3-(1-isopropyl-1H-imidazol-2-yl)-thiourea (15a).* Amine 14a (20 g, 0.16 mol) in CH2Cl2 (100 mL) was added dropwise to a solution of 3,4-dichlorophenylisothiocyanate (10, 32.0 g, 0.16 mol) in CH2Cl2 (75 mL). The mixture was stirred at rt for 18 h, and the solvent was removed under reduced pressure. The residue was suspended in Et2O and filtered to give a brown solid (22.6 g, 46%), mp 164−165 °C. ^{1}H NMR (CDCl3): δ 1.45 (d, $J = 6.7$ Hz, 6 H), 4.75 (m, 1 H), 6.73 (s, 1 H), 6.78 (s, 1 H), 7.03 (dd, $J = 2.2$ Hz, and 8.6 Hz, 1 H), 7.31 (d, $J = 8.6$ Hz, 1 H), 7.64 (br s,

1 H), 8.20 (d, J = 2.2 Hz, 1 H), 13.6 (br s, 1 H). MS (ESI): m/z 329 $[M + 1]^+$. Anal. ($C_{13}H_{14}Cl_2N_4S$): C, H, N, Cl, S.

Compounds **15b−d** were prepared by the same procedure, except, different amines **14b−d** were used in place of **14a**.

1-(3,4-Dichlorophenyl)-3-(1-tert-butyl-1H-imidazol-2-yl)-thiourea (**15b**). A brown crystalline solid prepared from compound **10** and amine **14b** in 74% yield, mp 175.4 °C (dec). ^{1}H NMR (CDCl$_3$): δ 1.62 (s, 9 H), 6.71 (br s, 1 H), 6.78 (br s, 1 H), 7.03 (d, J = 8.3 Hz, 1 H), 7.33 (d, J = 8.5 Hz, 1 H), 7.56 (br s, 1 H), 7.98 (s, 1 H). MS (ESI): m/z 343 $[M + 1]^+$.

1-(1-Benzyl-1H-imidazol-2-yl)-3-(3,4-dichlorophenyl)-thiourea (**15c**). An off-white solid prepared from compound **10** and amine **14c** in 23% yield, mp 163.5−164.5 °C. ^{1}H NMR (CDCl$_3$): δ 5.11 (s, 2 H), 6.60 (s, 1 H), 6.79 (s, 1 H), 7.14−7.38 (m, 6 H), 7.67 (s, 1 H), 7.96 (s, 1 H). MS (ESI): m/z 377 $[M + 1]^+$.

1-(3,4-Dichlorophenyl)-3-(1H-imidazol-2-yl)-thiourea (**15d**). A light-brown solid prepared from compound **10** and amine **14d** in 42% yield. ^{1}H NMR (CD$_3$OD): δ 6.90 (s, 2 H), 7.35 (d, J = 8.7 Hz, 1 H), 7.56 (dd, J = 2.4 and 8.7 Hz, 1 H), 8.04 (d, J = 2.4 Hz, 1 H). LC-MS (ESI): m/z 287 $[M + 1]^+$.

5. Synthesis of Compounds 16a−16h. *Synthesis of N-(3,4-dichlorophenyl)-N′-isobutyryl-N″-(1-isopropyl-1H-imidazol-2-yl)-guanidine* (**16a**). A solution of isobutyryl chloride (0.63 mL, 6.0 mmol) in CHCl$_3$ (10 mL) was added dropwise to a suspension of **8h** (1.05 g, 3.0 mmol) and triethylamine (1.4 mL, 10.0 mmol) in CHCl$_3$ (30 mL) at 0 °C. The reaction mixture was stirred at rt for 18 h. The solvent was removed under reduced pressure. The residue was purified via flash column chromatography (0−5% ethyl acetate in hexanes), followed by recrystallization in MeOH to give **16a** as a white crystalline solid (0.57 g, 73%), mp 9−92 °C. ^{1}H NMR (CD$_3$OD): δ 1.30 (d, J = 6.9 Hz, 6 H), 1.45 (d, J = 6.9 Hz, 6 H), 2.69 (m, 1 H), 4.76 (m, 1 H), 6.84 (s, 1 H), 6.94 (s, 1 H), 7.33 (dd, J = 2.1 and 8.7 Hz, 1 H), 7.44 (d, J = 8.7 Hz, 1 H), 8.39 (d, J = 2.1 Hz, 1 H). ^{13}C NMR (CDCl$_3$): δ 18.2, 19.0, 22.7, 24.3, 29.6, 34.0, 37.1, 46.0, 111.6, 112.8, 114.3, 119.3, 122.0, 123.7, 125.6, 129.9, 132.2, 138.0, 145.3, 148.1, 179.3. MS (ESI): m/z 382 $[M + 1]^+$. Anal. ($C_{17}H_{21}Cl_2N_5O$): C, H, N, Cl.

Compounds **16b−h** were prepared by the same procedure by treatment of **8h**, **8i**, or **8j** with appropriate acyl chloride or dialkyl-dicarbonate.

N-(3,4-Dichlorophenyl)-N′-(2-ethyl-butyryl)-N″-(1-isopropyl-1H-imidazol-2-yl) guanidine (**16b**). The title compound was prepared from **8h** and 2-ethylbutyryl chloride using the same procedure for the preparation of **16a** to give pale-brown solid (0.9 g, 75%), mp 57.5−59.0 °C. ^{1}H NMR (CDCl$_3$): δ 0.98 (t, J = 4.7 Hz, 6 H), 1.45 (d, J = 6.6 Hz, 6 H), 1.59−1.83 (m, 4 H), 2.23−2.32 (m, 1 H), 4.78 (m, 1 H), 6.77 (s, 1 H), 6.85 (s, 1 H), 7.24 (dd, J = 2.4 and 8.7 Hz, 1 H), 7.35 (d, J = 8.7 Hz, 1 H), 8.35 (d, J = 2.4 Hz, 1 H). ^{13}C NMR (CDCl$_3$): δ 11.4, 11.7, 22.7, 24.3, 25.2, 46.0, 52.5, 53.3, 111.5, 119.4, 122.0, 123.8, 125.6, 129.9, 132.2, 138.1, 145.2, 148.2, 178.5. MS (ESI): m/z 410 $[M + 1]^+$. Anal. ($C_{19}H_{25}Cl_2N_5O$): C, H, N, Cl.

N-(3,4-Dichlorophenyl)-N′-isobutyryl-N″-(1-isopropyl-1H-imidazol-2-yl)-guanidine (**16c**). The title compound was prepared from **8h** and isobutyryl chloride using the same procedure for the preparation of **16a** in 84% yield, mp 101−102 °C. ^{1}H NMR (CDCl$_3$): δ 1.37 (s, 9 H), 1.44 (d, J = 6.6 Hz, 6 H), 4.76 (m, 1 H), 6.77 (s, 1 H), 6.85 (s, 1 H), 7.24 (br d, J = 8.4 Hz, 1 H), 7.34 (d, J = 8.4 Hz, 1 H), 8.34 (br s, 1 H). ^{13}C NMR (CDCl$_3$): δ 22.7, 27.2, 40.7, 46.0, 111.6, 119.4, 122.1, 123.7, 125.6, 129.9, 132.2, 138.2, 145.6, 148.2, 181.5. MS (ESI): m/z 396 $[M + 1]^+$. Anal. ($C_{18}H_{23}Cl_2N_5O$): C, H, N, Cl.

N-(3,4-Dichlorophenyl)-N′-(1-isopropyl-1H-imidazol-2-yl)-N″-phenyl-acetyl-guanidine (**16d**). The title compound was prepared from **8h** and phenylacetyl chloride as that for the preparation of **16a**, giving a white solid in 85% yield, mp 91−92 °C. ^{1}H NMR (CDCl$_3$): δ 1.41 (d, J = 6.6 Hz, 6 H), 3.81 (s, 2 H), 4.73 (m, 1 H), 6.69 (s, 1 H), 6.72 (s, 1 H), 7.19 (dd, J = 1.9 and 8.8 Hz, 1 H), 7.30−7.40 (m, 6 H), 8.29 (br s, 1 H). ^{13}C NMR (CDCl$_3$): δ 22.7, 45.2, 46.0, 119.4, 122.1, 123.4, 125.7, 127.6, 128.8, 129.8,

129.9, 132.2, 133.0, 138.0, 173.6. MS (ESI): m/z 430 $[M + 1]^+$. Anal. ($C_{21}H_{21}Cl_2N_5O$): C, H, N, Cl.

N-(3,4-Dichlorophenyl)-N′-(t-butyloxycarbonyl)-N″-(1-isopropyl-1H-imidazol-2-yl)-guanidine (**16e**). A solution of di-tert-butyl dicarbonate (9.3 g, 42.6 mmol) in CHCl$_3$ (80 mL) was added dropwise with stirring to a mixture of **8h** (12.4 g, 35.5 mmol), triethylamine (14 mL, 0.10 mol), and DMAP (0.10 g, 0.8 mmol) in CHCl$_3$ (250 mL) at 0 °C. The reaction mixture was further stirred at rt for 18 h, and saturated aqueous Na$_2$CO$_3$ solution (100 mL) was added. The organic layer was separated, and the aqueous layer was extracted with CHCl$_3$ (80 mL × 2). The CHCl$_3$ extracts were combined, washed with brine, dried over Na$_2$SO$_4$, and evaporated to dryness under the reduced pressure to give a light-brown gum. Purification was achieved by the use of flash chromatography (0−15% ethyl acetate in hexanes), followed by recrystallization from MeOH to give **16e** as a white solid (10 g, 68% yield), mp 101−102 °C. ^{1}H NMR (CDCl$_3$): δ 1.43 (d, J = 6.6 Hz, 6 H), 1.55 (s, 9 H), 4.74 (m, 1 H), 6.76 (d, J = 1.5 Hz, 1 H), 6.86 (d, J = 1.5 Hz, 1 H), 7.25 (dd, J = 2.4 and 8.7 Hz, 1 H), 7.35 (d, J = 8.7 Hz, 1 H), 8.32 (d, J = 2.4 Hz, 1 H), 10.2 (s, 1 H), 12.9 (br s, 1 H). ^{13}C NMR (CDCl$_3$): δ 22.8, 28.1, 45.9, 82.8, 111.6, 119.3, 121.9, 123.7, 125.5, 129.9, 132.2, 138.2, 144.9, 148.2, 153.9. MS (ESI): m/z 412 $[M + 1]^+$. Anal. ($C_{18}H_{23}Cl_2N_5O_2$): C, H, N, Cl.

N-(1-tert-Butyl-1H-imidazol-2-yl)-N′-(3,4-dichlorophenyl)-N″-(2,2-dimethylpropionyl)-guanidine (**16f**). The title compound was prepared from **8i** and 2,2-dimethylpropionyl chloride as that for the preparation of **16a** to give a white crystalline solid (0.95 g, 42%), mp 193.1−193.8 °C. ^{1}H NMR (CD$_3$OD): δ 1.34 (s, 9 H), 1.67 (s, 9 H), 6.74 (d, J = 1.3 Hz, 1 H), 6.94 (d, J = 1.3 Hz, 1 H), 7.24 (dd, J = 2.4 and 8.7 Hz, 1 H), 7.44 (d, J = 8.7 Hz, 1 H), 8.32 (d, J = 2.4 Hz, 1 H). ^{13}C NMR (CD$_3$OD): δ 27.2, 29.6, 56.0, 115.0, 121.8, 121.9, 123.2, 125.4, 130.9, 131.5, 138.4, 145.4, 148.4, 181.1. MS (ESI): m/z 410 $[M + 1]^+$. Anal. ($C_{19}H_{25}Cl_2N_5O$): C, H, N, Cl.

N-(1-tert-Butyl-1H-imidazol-2-yl)-N′-(3,4-dichlorophenyl)-N″-(2-ethylbutyryl)-guanidine (**16g**). The title compound was prepared from **8i** and 2-ethylbutyryl chloride as colorless oil (1.4 g, 60% yield), using the same procedure for the preparation of **16a**. ^{1}H NMR (CD$_3$OD): δ 0.99 (t, J = 7.4 Hz, 6 H), 1.67 (s, 9 H), 1.77 (m, 4 H), 2.26 (m, 1 H), 6.75 (d, J = 1.6 Hz, 1 H), 7.79 (d, J = 1.6 Hz, 1 H), 7.20 (dd, J = 2.5 and 8.7 Hz, 1 H), 7.34 (d, J = 8.7 Hz, 1 H), 8.19 (d, J = 2.5 Hz, 1 H). ^{13}C NMR (CD$_3$OD): δ 11.8, 25.1, 29.6, 52.6, 55.9, 113.5, 120.5, 121.8, 123.2, 126.3, 130.0, 132.4, 138.0, 145.0, 149.0, 178.6. MS (ESI): m/z 424 $[M + 1]^+$. Anal. ($C_{20}H_{27}Cl_2N_5O$): C, H, N, Cl.

N-(1-Benzyl-1H-imidazol-2-yl)-N′-(3,4-dichlorophenyl)-N″-isobutyryl-guanidine (**16h**). The title compound was prepared by treatment of **8j** with isobutyryl chloride in 33% yield as white needles (0.50 g, 33%), mp 117.5−118.5 °C. ^{1}H NMR (CDCl$_3$): δ 1.33 (d, J = 6.9 Hz, 6 H), 2.71 (d, J = 6.9 Hz, 6 H), 5.18 (s, 2 H), 6.72 (d, J = 1.2 Hz, 1 H), 6.88 (d, J = 1.2 Hz, 1 H), 7.18−7.34 (m, 7 H), 8.09 (s, 1 H), 10.85 (s, 1 H), 13.84 (s, 1 H). ^{13}C NMR (CDCl$_3$): δ 19.1, 37.3, 48.2, 116.0, 119.7, 122.1, 124.0, 125.9, 127.0, 127.6, 128.7, 130.0, 132.2, 137.2, 137.8, 145.8, 149.2, 179.5. MS (ESI): m/z 430 $[M + 1]^+$. Anal. ($C_{21}H_{21}Cl_2N_5O_2$): C, H, N, Cl.

6. Synthesis of 1-Isopropyl-4,5-dihydro-1H-imidazol-2-ylamine (17)[26]. A solution of cyanogen bromide (10.6 g, 0.10 mol) in MeOH (20 mL) was added dropwise to a solution of N-isopropyl-ethylenediamine (10.3 g, 0.10 mol) in MeOH (10 mL) at 0 °C. The reaction mixture was then heated under reflux for 1 h. The solvent was removed under reduced pressure. The crude product was suspended in diethyl ether, filtered, and washed with diethyl ether to give **17** as a yellow solid (20.0 g, 96% yield), mp 150.5−152.5 °C. ^{1}H NMR (CDCl$_3$): δ 1.26 (d, J = 6.6 Hz, 6 H), 3.59−3.71 (m, 4 H), 4.39 (m, 1 H), 7.57 (br s, 1 H), 7.71 (br s, 2 H). MS (EI): m/z 127 $[M^+]$.

7. Synthesis of 1-(3,4-dichlorophenyl)-3-(1-isopropyl-4,5-dihydro-1H-imidazol-2-yl)-thiourea (18) and 2-Imino-3-isopropyl-imidazolidine-1-carbothioic Acid (3,4-Dichloro-phenyl)-amide (18′). Amine **17** (5.2 g, 25 mmol) was added to a

stirring solution of 3,4-dichlorophenyl isothiocyanate (4.08 g, 20.0 mmol) in CH$_2$Cl$_2$ (80 mL), followed by addition of triethylamine (4.2 mL, 30 mmol) at ambient temperature. The reaction mixture was further stirred at rt for 72 h. The reaction mixture was basified with aqueous saturated Na$_2$CO$_3$ solution. The organic phase was separated, washed with brine, dried over Na$_2$SO$_4$, and filtered, and the solvent was removed under reduced pressure to give a yellow solid. The crude product was purified via column chromatography (silica gel, CH$_2$Cl$_2$) to give two white crystalline solids **18** and **18′**. The former is a less polar isomer (27% yields, mp 184.0−185.0 °C), and the latter is a more polar isomer (50% yields, mp 134.0−135.0 °C). The structure of **18** was confirmed by single crystal X-ray diffraction spectrum. Both isomers gave the same MS (ESI): m/z 331 [M + 1]$^+$. ^{1}H NMR (CDCl$_3$) of **18**: δ 1.21 (d, J = 6.6 Hz, 6 H), 3.48−3.55 (m, 2 H), 3.70−3.76 (m, 2 H), 4.39 (m, 1 H), 7.05 (br s, 1 H), 7.32 (d, J = 8.7 Hz, 1 H), 8.03 (s, 1 H). ^{1}H NMR (CDCl$_3$) of **18′**: δ 1.24 (d, J = 6.6 Hz, 6 H), 3.45 (t, J = 7.8 Hz, 2 H), 3.72 (m, 1 H), 4.28 (t, J = 7.8 Hz, 2 H), 7.39 (d, J = 8.7 Hz, 1 H), 7.54 (dd, J = 1.8 and 8.7 Hz, 1 H), 7.86 (d, J = 1.8 Hz, 1 H).

8. Synthesis of Compounds 8a−f. *Synthesis of N-(3,4-dichlorophenyl)-N′-isopropyl-N″-(1-isopropyl-1H-imidazol-2-yl)-guanidine (8a)*[28]*.* Triethylamine (35.0 mL, 0.24 mol) was added dropwise to a stirring suspension of amine **14a** (3.0 g, 24.0 mmol), thiourea **11a** (6.3 g, 24 mmol), and HgO (7.8 g, 36.0 mmol) in DMF (∼100 mL) at ambient temperature. The mixture was further stirred at rt for 60 h. The precipitate was filtered through a pad of Celite. The filtrate was concentrated under reduced pressure. Water was added to the residue, and the mixture was extracted with chloroform. The organic extracts were combined, washed with brine, dried over Na$_2$SO$_4$, and filtered, and the solvent was removed under reduced pressure. The crude product was purified via column chromatography (silica gel, 0−33% ethyl acetate in hexanes) to give a light-brown oil, which was recrystallized in MeOH/CHCl$_3$ mixed solvent to give **8a** as a light-yellow crystalline solid (1.3 g, 31% yield), mp 111−112 °C. ^{1}H NMR (CDCl$_3$ w/TFA-*d*): δ 1.34 (d, J = 6.6 Hz, 6 H), 1.41 (d, J = 6.6 Hz, 6 H), 4.05 (m, 1 H), 4.52 (m, 1 H), 6.92 (d, J = 2.4 Hz, 1 H), 7.05 (dd, J = 2.4 and 8.4 Hz, 1 H), 7.13 (d, J = 2.4 Hz, 1 H), 7.32 (d, J = 2.4 Hz, 1 H), 7.41 (d, J = 8.4 Hz, 1 H). ^{13}C NMR (CDCl$_3$): δ 22.7, 42.9, 45.1, 110.4, 122.7, 123.2, 125.6, 128.0, 130.9, 133.0, 138.0, 149.8, 150.5. MS [ESI]: m/z 354 [M + 1]$^+$. Anal. (C$_{16}$H$_{21}$Cl$_2$N$_5$): C, H, N, Cl.

Compounds **8b−f** was prepared by the same procedure except different compounds **11** and **14** were used.

N-tert-Butyl-N′-(3,4-dichlorophenyl)-N″-(1-isopropyl-1H-imidazol-2-yl)-guanidine (8b). Compound **8b** was prepared by the same procedure from **14a** and **11c** to give a light-yellow crystalline solid in 40% yield, mp 57.2 °C. ^{1}H NMR (CDCl$_3$): δ 1.38 (d, J = 6.7 Hz, 6 H), 1.44 (s, 9 H), 4.10 (br s, 1 H), 4.76 (m, 1 H), 6.68 (d, J = 2.4 Hz, 1 H), 6.75 (d, J = 2.4 Hz, 1 H), 7.06 (d, J = 8.4 Hz, 1 H), 7.32 (s, 1 H), 7.38 (d, J = 8.4 Hz, 1 H), 11.67 (br s, 1 H). ^{13}C NMR (CDCl$_3$): δ 22.1, 29.3, 46.7, 52.6, 110.4, 113.5, 121.3, 122.2, 126.8, 130.3, 132.1, 138.4, 144.5, 155.6. MS (ESI): m/z 368 [M + 1]$^+$. Anal. (C$_{17}$H$_{23}$Cl$_2$N$_5$): C, H, N, Cl.

N-(3,4-Dichlorophenyl)-N′-(2,2-dimethylpropyl)-N″-(1-isopropyl-1H-imidazol-2-yl)-guanidine Hydrochloride (8c). Compound **8c** was prepared by the same procedure from **14a** and **11b** to give a white crystalline solid in 31% yield, mp 139.8 °C. ^{1}H NMR (CDCl$_3$): δ 1.05 (s, 9 H), 1.46 (d, J = 6.7 Hz, 6 H), 3.14 (d, J = 5.9 Hz, 2 H), 4.64 (m, 1 H), 6.55 (d, J = 2.4 Hz, 1 H), 6.72 (d, J = 2.4 Hz, 1 H), 7.23 (d, J = 8.1 Hz, 2 H), 7.37 (s, 1 H), 7.60 (br s, 1 H), 10.16 (br s, 1 H). ^{13}C NMR (CDCl$_3$): δ 22.1, 27.4, 32.3, 46.9, 54.1, 110.8, 113.7, 121.2, 122.3, 127.0, 130.3, 132.1, 138.2, 144.7, 156.64. MS (ESI): m/z 382 [M + 1]$^+$. Anal. (C$_{18}$H$_{26}$Cl$_3$N$_5$): C, H, N, Cl.

N-tert-Butyl-N′-(3,4-dichlorophenyl)-N″-[1-(2,2-dimethyl-propyl)-1H-imidazol-2-yl]-guanidine (8d). Compound **8d** was prepared by the same procedure from **14c** and **11c** to give a light-brown crystalline solid in 44% yield, mp 124.8 °C. ^{1}H NMR (CDCl$_3$): δ 0.97 (s, 9 H), 1.45 (s, 9 H), 3.77 (s, 2 H), 4.14 (br s, 1 H), 6.62 (d, J = 2.4 Hz, 1 H), 6.74 (d,

J = 2.4 Hz, 1 H), 7.06 (d, J = 8.5 Hz, 1 H), 7.29 (s, 1 H), 7.47 (d, J = 8.5 Hz, 1 H). ^{13}C NMR (CDCl$_3$): δ 27.9, 29.7, 33.1, 51.4, 55.0, 116.0, 122.2, 123.0, 125.4, 127.7, 130.9, 133.1, 138.7, 149.8, 152.1. MS (ESI): m/z 396 [M + 1]$^+$. Anal. (C$_{19}$H$_{27}$Cl$_2$N$_5$): C, H, N, Cl.

N-(3,4-Dichlorophenyl)-N′-[1-(2,2-dimethyl-propyl)-1H-imidazol-2-yl]-N″-isopropyl-guanidine (8e). Title compound was prepared from **14c** and **11a** as a white crystalline solid in 36% yield, mp 89.5 °C. ^{1}H NMR (CDCl$_3$): δ 0.99 (s, 9 H), 1.23 (s, 6 H), 3.77 (s, 2 H), 4.04 (br s, 1 H), 4.20 (m, 1 H), 6.63 (d, J = 2.4 Hz, 1 H), 6.97 (d, J = 2.4 Hz, 1 H), 7.20 (d, J = 8.4 Hz, 1 H), 7.40 (d, J = 8.4 Hz, 2 H). ^{13}C NMR (CDCl$_3$): δ 23.0, 28.0, 33.3, 42.9, 55.2, 116.1, 122.2, 123.3, 125.8, 128.1, 131.0, 133.2, 138.3, 149.9, 152.1. MS (ESI): m/z 382 [M + 1]$^+$. Anal. (C$_{18}$H$_{25}$Cl$_2$N$_5$): C, H, N, Cl.

N-(1-tert-Butyl-1H-imidazol-2-yl)-N′-(3,4-dichlorophenyl)-N″-isopro-pyl-guanidine Hydrochloride (8f). Prepared from **14b** and **11a** as a white crystalline solid in 54% yield, mp 180.7 °C. ^{1}H NMR (CD$_3$OD): δ 1.26 (d, J = 6.5 Hz, 6 H), 1.67 (s, 9 H), 4.07 (m, 1 H), 6.80 (d, J = 1.8 Hz, 1 H), 7.06 (d, J = 1.8 Hz, 1 H), 7.09 (dd, J = 2.1 and 8.7 Hz, 1 H), 7.34 (d, J = 2.1 Hz, 1 H), 7.45 (d, J = 8.7 Hz, 1 H). ^{13}C NMR (CDCl$_3$): δ 22.7, 28.3, 44.6, 57.8, 112.1, 112.3, 121.1, 122.0, 126.8, 130.2, 131.9, 138.1, 145.1, 154.4. MS (ESI): m/z 368 [M + 1]$^+$. Anal. (C$_{17}$H$_{24}$Cl$_3$N$_5$): C, H, N, Cl.

9. General Procedure for the Synthesis of Compound 8g−m. *N-(3,4-Dichlorophenyl)-N′-(1-isopropyl-1H-imidazol-2-yl)-piperidinecarboxamidine Hydrochloride Salt (8g)*. HgCl$_2$ (1.6 g, 6.0 mmol) was added at rt to a stirring solution consisting of **15a** (1.0 g, 3.0 mmol), piperidine (2.0 mL, 20.0 mmol), and acetonitrile (50 mL). After the addition, the reaction mixture was further stirred at rt for 18 h and then filtered through a pad of Celite. The filtrate was concentrated under reduced pressure. The crude product was purified by a silica gel column and eluted with CHCl$_3$ to give a gummy yellow solid (0.80 g, 64% yield). The pure **8g** was obtained as a yellow crystalline solid by recrystallization from MeOH, mp 99.0−101.0 °C. ^{1}H NMR (CD$_3$OD): δ 1.40 (d, J = 6.6 Hz, 6 H), 1.74 (br s, 6 H), 3.67 (br s, 4 H), 4.56 (m, 1 H), 6.69 (s, 1 H), 6.92 (s, 1 H), 6.95 (dd, J = 2.4 and 8.5 Hz, 1 H), 7.16 (d, J = 2.4 Hz, 1 H), 7.35 (d, J = 8.5 Hz, 1 H). ^{13}C NMR (CD$_3$OD): δ 22.3, 25.5, 27.2, 113.9, 114.5, 121.5, 122.9, 127.8, 132.1, 133.6, 140.7, 146.9, 156.1. MS (ESI): m/z 380 [M + 1]$^+$. Anal. (C$_{18}$H$_{24}$Cl$_3$N$_5$): C, H, N, Cl.

N-(3,4-Dichlorophenyl)-N′-(1-isopropyl-1H-imidazol-2-yl)-guanidine Hydrochloride Salt (8h). Mercuric chloride (HgCl$_2$, 2.4 g, 9.0 mmol) was added with stirring to a solution of **15a** (1.5 g, 4.5 mmol) in methanolic NH$_3$ (7 N NH$_3$ in MeOH, 5.0 mL, 35.0 mmol) and DMF (70 mL) at ambient temperature. The reaction mixture was further stirred at rt for 18 h. The precipitate was filtered off through a pad of Celite. The filtrate was concentrated under reduced pressure. The residue was suspended in CH$_2$Cl$_2$, filtered, and washed with CH$_2$Cl$_2$ to give a pale-brown solid, which was recrystallized from MeOH to give **8h** as a white crystalline solid (0.70 g, 50% yield), mp 277.0−277.9 °C. ^{1}H NMR (CD$_3$OD): δ 1.44 (d, J = 6.6 Hz, 6 H), 4.56 (m, 1 H), 7.04 (d, J = 2.4 Hz, 1 H), 7.18 (d, J = 2.4 Hz, 1 H), 7.30 (dd, J = 2.7 and 8.7 Hz, 1 H), 7.45 (d, J = 8.7 Hz, 1 H), 7.80 (d, J = 2.7 Hz, 1 H). ^{13}C NMR (CD$_3$OD): δ 22.3, 115.0, 115.6, 122.0, 124.0, 128.0, 131.7, 133.4, 140.1, 147.4, 156.7. MS (ESI): m/z 312 [M + 1]$^+$. Anal. (C$_{13}$H$_{16}$Cl$_3$N$_5$): C, H, N, Cl.

N-(1-tert-Butyl-1H-imidazol-2-yl)-N′-(3,4-dichlorophenyl)-guanidine HCl Salt (8i). The title compound was prepared by the same procedure except **15b** was used in place of **15a** to give a bright-white crystalline solid (1.2 g, 63%), mp 264.7 °C. ^{1}H NMR (CD$_3$OD): δ 1.63 (s, 9 H), 7.00 (d, J = 2.5 Hz, 1 H), 7.17 (d, J = 2.5 Hz, 1 H), 7.29 (dd, J = 2.4, and 8.7 Hz, 1 H), 7.45 (d, J = 8.7 Hz, 1 H), 7.81 (d, J = 2.4 Hz, 1 H). ^{13}C NMR (DMSO-d$_6$): δ 28.8, 58.2, 113.9, 115.9, 119.9, 121.0, 124.4, 131.0, 131.3, 140.0, 146.5, 154.5. MS (ESI): m/z 326 [M + 1]$^+$. Anal. (C$_{14}$H$_{18}$Cl$_3$N$_5$): C, H, N, Cl.

N-(Benzyl-1H-imidazol-2-yl)-N′-(3,4-dichlorophenyl)guanidine Hydro-chloride (8j). The title compound was prepared by the same procedure,

except **15c** was used in place of **15a** to give an off-white solid (1.8 g, 85% yield), mp 237−238 °C. ^{1}H NMR (CD$_3$OD): δ 5.10 (s, 2 H), 7.04 (d, J = 2.4 Hz, 1 H), 7.10 (d, J = 2.4 Hz, 1 H), 7.24 (dd, J = 2.4 and 8.7 Hz, 1 H), 7.25−7.40 (m, 6 H), 7.71 (d, J = 2.4 Hz, 1 H). ^{13}C NMR (CD$_3$-OD): δ 115.4, 118.6, 122.0, 124.0, 128.0, 129.0, 129.5, 130.2, 131.6, 133.4, 137.1, 139.9, 148.4, 156.8. MS (ESI): m/z 360 [M + 1]$^+$. Anal. (C$_{17}$H$_{16}$Cl$_3$N$_5$): C, H, N, Cl.

*N-(1-Benzyl-1H-imidazol-2-yl)-N′-(3,4-dichlorophenyl)-N″-isopropyl-guanidine (**8k**).* The title compound was prepared by the same procedure, except **15c** and isopropylamine instead of ammonia were used to give an off-white solid (2.5 g, 61%), mp 87−89 °C. ^{1}H NMR (CDCl$_3$ w/TFA): δ 1.23 (d, J = 6.6 Hz, 6 H), 4.03 (m, 1 H), 5.02 (s, 2 H), 6.39 (s, 1 H), 6.55 (s, 1 H), 6.77 (dd, J = 2.4 and 8.7 Hz, 1 H), 7.04−7.13 (m, 2 H), 7.26−7.29 (m, 2 H), 7.35−7.44 (m, 3 H). ^{13}C NMR (CDCl$_3$): δ 22.9, 43.0, 47.8, 114.5, 123.2, 123.5, 126.0, 127.4, 127.6, 128.6, 131.1, 133.3, 138.0, 138.4, 150.3, 151.6. MS (ESI): m/z 402 [M + 1]$^+$. Anal. (C$_{20}$H$_{21}$Cl$_2$N$_5$): C, H, N, Cl.

*Synthesis of N-(3,4-dichlorophenyl)-N′-(1H-imidazol-2-yl)-guanidine Hydrochloride (**8l**).* Mercuric chloride (1.6 g, 6.0 mmol) was added with stirring at rt to a mixture of **15d** (1.15 g, 4.0 mmol) and methanolic ammonia (7 N, 5 mL, 35 mmol) in DMF (40 mL). The mixture was further stirred at rt for 24 h and filtered through Celite. The filtrate was evaporated to dryness under reduced pressure. The crude product was suspended in ethyl acetate and filtered to give a light-brown solid, which was recrystallized successively in MeOH and water to give **8l** as an off-white solid (0.44 g, 21% yield), mp 240 °C (decd). ^{1}H NMR (CD$_3$OD): δ 6.97 (s, 2 H), 7.31 (dd, J = 2.4 and 8.7 Hz, 1 H), 7.43 (d, J = 8.7 Hz, 1 H), 7.75 (d, J = 2.4 Hz, 1 H). ^{13}C NMR (CD$_3$OD): δ 115.9, 122.0, 123.9, 127.9, 131.5, 133.3, 139.9, 148.9, 156.6. MS (ESI): m/z 270 [M + 1]$^+$. Anal. (C$_{10}$H$_{10}$Cl$_3$N$_5$): C, H, N, Cl.

*Synthesis of N-(3,4-dichlorophenyl)-N′-(1H-imidazol-2-yl)-N″-iso-propyl-guanidine (**8m**).* Thiourea **15d** (1.0 g, 3.5 mmol) was suspended in 10.0 mL of acetonitrile and to the suspension, dicyclohexylcarbodi-imide (DCC) (1.0 g, 5.2 mmol), isopropyl amine (0.60 mL, 6.9 mmol), and a catalytic amount of *i*-Pr$_2$EtN (0.12 mL) were added successively. The reaction mixture was then stirred at ambient temperature for 5 h. The thickened suspension was evaporated under reduced pressure to dryness and extracted with EtOAc 100 mL × 3. The organic extracts were combined, washed with brine, dried over Na$_2$SO$_4$, and concentrated under reduced pressure. The residue was purified via flash chromatography (silica gel, 5% ethyl acetate in hexanes) to give a white solid, which was recrystallized from methanol to give **8m** as a white crystalline solid (0.70 g, 64%), mp 163.0 °C. ^{1}H NMR (CD$_3$OD): δ 1.32 (d, J = 6.6 Hz, 6 H), 4.13 (m, 1 H), 6.97 (s, 2 H), 7.14 (dd, J = 2.4 and 8.7 Hz, 2 H), 7.38 (d, J = 2.4 Hz, 1 H), 7.49 (d, J = 8.7 Hz, 1 H). ^{13}C NMR (CD$_3$OD): δ 23.6, 45.0, 118.4, 122.5, 124.3, 126.8, 131.6, 133.3, 141.4, 152.4, 153.3. MS (ESI): m/z 312 [M + 1]$^+$. Anal. (C$_{13}$H$_{15}$Cl$_2$N$_5$): C, H, N, Cl.

10. General Procedure for the Synthesis of Compounds 9a−9e.

*Synthesis of N-(3,4-dichlorophenyl)-N′-(1-isopropyl-4,5-dihydro-1H-imidazol-2-yl)-guanidine Hydrochloride (**9a**).* Mercuric chloride (0.49 g, 1.8 mmol) was added in small portions at rt to a stirring solution of **18** (0.41 g, 1.2 mmol) in methanolic NH$_3$ (7 N NH$_3$ in MeOH, 1.0 mL, 7.0 mmol) and DMF (15 mL) mixed solvents. After the addition, the mixture was stirred at rt for additional 30 min. The precipitate was filtered off through a pad of Celite. The filtrate was concentrated in vacuo, and the solid material was suspended in CH$_2$Cl$_2$, filtered, and washed with CH$_2$Cl$_2$ to give **9a** as a white solid which was recrystallized from MeOH to give a colorless crystalline solid (0.75 g, 71% yield), mp 259.5−260.5 °C. ^{1}H NMR (CD$_3$OD): δ 1.21 (d, J = 6.6 Hz, 6 H), 3.66 (s, 4 H), 4.21 (m, 1 H), 7.23 (dd, J = 2.4 and 8.5 Hz, 1 H), 7.47 (d, J = 8.5 Hz, 1 H), 7.70 (d, J = 2.4 Hz, 1 H). ^{13}C NMR (CD$_3$-OD): δ 19.3, 44.7, 120.2, 121.4, 124.8, 130.5, 130.9, 138.7, 156.1, 160.0. MS (ESI): m/z 314 [M + 1]$^+$. Anal. (C$_{13}$H$_{18}$Cl$_3$N$_5$): C, H, N, Cl.

*Synthesis of N-(3,4-dichlorophenyl)-N′-isopropyl-N″-(1-isopropyl-4,5-dihydro-1H-imidazol-2-yl)-guanidine Hydrochloride (**9b**).* Mercuric chloride (2.0 g, 7.4 mmol) was added with stirring to a mixture of **18** (1.25 g, 3.7 mmol) and isopropylamine (0.80 mL, 9.3 mmol) in CH$_3$CN (80 mL) at ambient temperature. The reaction mixture was stirred at rt for additional 5 min, filtered through Celite, and concentrated under reduced pressure to dryness. The product was purified via column chromatography (silica gel, 0−5% CH$_3$OH in CH$_2$Cl$_2$), followed by recrystallization from CH$_2$Cl$_2$/hexanes to give **9b** as a white solid (0.85 g, 58% yield), mp 156.5−157.5 °C. ^{1}H NMR (CDCl$_3$): δ 1.18 (d, J = 6.9 Hz, 6 H), 1.27 (d, J = 6.6 Hz, 6 H), 3.37 (s, 4 H), 3.87 (m, 1 H), 4.24 (m, 1 H), 7.10 (dd, J = 2.1 and 8.4 Hz, 1 H), 7.24 (d, J = 8.4 Hz, 1 H), 7.30 (d, J = 2.1 Hz, 1 H). ^{13}C NMR (CDCl$_3$): δ 19.6, 22.4, 40.2, 40.4, 44.5, 44.7, 121.4, 122.9, 127.1, 130.0, 132.0, 137.6, 157.1, 157.9. MS (ESI): m/z 356 [M + 1]$^+$. Anal. (C$_{16}$H$_{24}$Cl$_3$N$_5$): C, H, N, Cl.

Compounds **9c−e** was synthesized by the same procedure for the preparation of compound **9b**, except *t*-butylamine, 1-adamantylamine, and piperidine were used in place of isopropylamine.

*N-tert-Butyl-N′-(3,4-dichlorophenyl)-N″-(1-isopropyl-4,5-dihydro-1H-imidazol-2-yl)-guanidine Hydrochloride (**9c**).* A white solid in 49% yield, mp 212.0−213.5 °C. ^{1}H NMR (CDCl$_3$): δ 1.21 (d, J = 6.6 Hz, 6 H), 1.46 (s, 9 H), 3.10−3.20 (m, 2 H), 3.28−3.34 (m, 2 H), 4.27 (m, 1 H), 7.17 (d, J = 8.4 Hz, 1 H), 7.25 (br s, 1 H), 7.31 (d, J = 8.4 Hz, 1 H). ^{13}C NMR (CDCl$_3$): δ 19.8, 29.2, 40.3, 40.4, 44.6, 53.1, 121.9, 123.1, 127.4, 130.4, 132.2, 137.8, 156.6, 158.4. MS (ESI): m/z 370 [M + 1]$^+$. Anal. (C$_{17}$H$_{26}$Cl$_3$N$_5$): C, H, N, Cl.

*N-Adamantan-1-yl-N′-(3,4-dichlorophenyl)-N″-(1-isopropyl-4,5-dihydro-1H-imidazol-2-yl)-guanidine Hydrochloride (**9d**).* A white solid in 55% yield, mp 240.5−241.5 °C. ^{1}H NMR (CD$_3$OD): δ 1.25 (d, J = 6.6 Hz, 6 H), 1.68−1.79 (m, 6 H), 2.11 (br s, 1 H), 3.22−3.34 (m, 2 H), 3.46 (t, J = 8.7 Hz, 2 H), 4.28 (m, 1 H), 7.02 (br d, J = 8.4 Hz, 1 H), 7.25 (s, 1 H), 7.51 (d, J = 8.4 Hz, 1 H). ^{13}C NMR (CD$_3$OD): δ 20.2, 31.1, 37.5, 41.6, 41.9, 42.7, 46.6, 54.8, 123.0, 124.5, 129.0, 132.3, 133.8, 139.4, 157.5, 159.1. MS (ESI): m/z 448 [M + 1]$^+$. Anal. (C$_{23}$H$_{32}$Cl$_3$N$_5$): C, H, N, Cl.

*N-(3,4-Dichlorophenyl)-N′-(1-isopropyl-4,5-dihydro-1H-imidazol-2-yl)-piperidine-1-carboxamidine Hydrochloride (**9e**).* A white solid in 56% yield, mp 110.0−110.6 °C. ^{1}H NMR (CDCl$_3$): δ 1.13 (d, J = 6.6 Hz, 6 H), 1.78 (br s, 6 H), 3.22 (br s, 4 H), 3.76 (br s, 4 H), 4.16 (m, 1 H), 7.27 (d, J = 2.4 Hz, 1 H), 7.31 (d, J = 8.4 Hz, 1 H), 7.38 (dd, J = 2.4 and 8.4 Hz, 1 H). ^{13}C NMR (CDCl$_3$): δ 19.7, 23.9, 25.6, 40.1, 40.4, 44.4, 47.9, 121.2, 122.1, 126.5, 130.2, 131.8, 139.0, 158.0, 158.1. MS (ESI): m/z 382 [M + 1]$^+$. Anal. (C$_{18}$H$_{25}$Cl$_2$N$_5$): C, H, N, Cl.

Antimalarial Studies. *1. Assessment of Metabolic Stability.* All samples were tested in human and mouse liver microsomal preparations, and selected samples were further tested in Rhesus monkey microsomes. Sample stocks at 10 or 20 mM (depending on solubility) in DMSO were diluted to a final concentration of 1 μM into a mixture containing 0.5 mg/mL of prewarmed pooled human or mouse liver microsomes (BD Gentest), 1.3 mM NADP (Sigma), 3.3 mM MgCl$_2$ (Sigma), and 0.1 M pH 7.4 PBS using a TECAN Genesis robotic liquid handler. The reaction was started with the addition of 1U/mL glucose-6-phosphate dehydrogenase G6PD. The mixture was incubated on a shaking platform at 37 °C, and aliquots were taken and quenched with the addition of an equal volume of cold acetonitrile at 0, 10, 20, 30, and 60 min. Samples were centrifuged at 3700 rpm for 10 min at 20 °C to remove debris. Sample quantification was carried out by LC/MS, and metabolic half-life was calculated by log plots of the total ion chromatograph area remaining. The results are shown in Table 1.

2. Metabolites Identification. Good antimalarial activity in mouse and Rhesus test models coupled with metabolic instability of **8a** and **8h** suggested that metabolite(s), not the parent drug, is the active species of the imidazole antimalarial. The metabolite identification of compound **8a** was performed in mice given a single oral dose of 50 mg/kg. The

Table 1. Metabolic Stability and Antimalarial Activities of the New Compounds

compd no.	metabolic stability ($t_{1/2}$, min)			P. falciparum IC_{50} (μg/mL)			EE P. berghei, dose (mg/kg/day)[b]	
	mouse	human	Rhesus	D6	TM[a]	W2	160	320
8a	5.9	22.5	7.8	2.6	2.4	0.8	CP (1/5), DP[d] (3/5)	CP[c] (4/5), DP (1/5)
8b	18.2	40.4		1.1		1.0	IA	IA
8c	10.8	32.9		1.4		0.9	IA	IA
8d	11.7	12.1		0.8		0.8	IA	IA
8e		20.3		1.1		1.5	IA	IA
8f	9	48		1.4	0.9	0.4	toxic	toxic
8g	19.7	27	7.8	0.5	0.3	0.3	IA	IA
8h	27.7	>60	>60	2.8	2.4	0.7	CP (5/5)	CP (5/5)
8i	48	>60	45	1.6	1.2	0.4	IA	IA
8j	37	>60		>2.0	>2.0	>2.0	IA	IA
8k	18	16		1.3	2.5	1.6	IA	IA
8l	23.6	42.5		0.5	2.5	1.4	IA	IA
8m	37.6	>60		>5.0	>5.0	0.6	toxic death (1/5)	toxic death (4/5)
9a	>60	>60	36.3	1.4	1.2	0.7	CP (4/5)	
9b	13.6	50.7		0.5	0.7	0.4	toxic and IA	toxic death
9c	16.6	>60		0.4		0.4	toxic and IA	toxic death
9d	>60	>60		0.3	0.2	0.2		
9e	21.5	>60		0.4	0.3	0.2	toxic	toxic death
16a	>60	>60		2.5	2.2	1.0	DP (2/5) IA (3/5)	CP (4/5), DP (1/5)
16b	>60	>60		1.0	2.9	0.3	IA	IA
16c	>60	>60		>5.0	>5.0	>5.0	IA	IA
16d	50.8	>60		>5.0	>5.0	>5.0	IA	IA
16e	>60	>60		>5.0	>5.0	>5.0	DP	CP (3/5), DP (2/5)
16f	>60	>60		>5.0	>5.0	>5.0	DP	DP
16g	12.5	26		>5.0	>5.0	>5.0	IA	DP
16h	11	19.7		>2.0	>2.0	>2.0	IA	IA

[a] TM = TM91C235. [b] Treatment for 3 days, on day before (−1), the day (0), and day after (1) sporozoites inoculation; CP = causal prophylaxis; DP = delayed patency; IA = inactive, [c] causal prophylaxis, [d] delay parasitemia patency.

blood and liver samples were collected from hours 1 to 32, and the parent and putative metabolites were analyzed by LC/MS in full scan mode (m/z 150−650) on an LTQ (Thermo Fisher Scientific, Bremen, Germany), with a data dependent MS^2 and MS^3 scan event on the most intense ions. A total of 19 and 17 putative metabolites were detected in mouse liver and blood samples, respectively. All metabolites detected in both blood and liver samples have the same retention time in LC chromatogram and m/z, except peak m/z 349 was observed only in blood samples not in liver homogenate. On the contrary, peak m/z 339 was detected only in liver samples not in blood. The major metabolites in both blood and liver samples are m/z 370, 384, 386, and 388.

3. In Vitro Antimalarial Activity against P. falciparum. The in vitro assays were conducted by using a modification of the semiautomated microdilution techniques of Desjardins et al. and Chulay et al.[29,30] 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.[31] The results are shown in Table 1.

4. 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.[16−21] Briefly, each compound was ground with a mortar and pestle and suspended in hydroxyethylcellulose and Tween 80 for compounds to be administered po, and those given sc were suspended in peanut oil. Each compound was

prepared at different dose levels. Compounds were administered either po or sc to mice once a day for three consecutive days, 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 any biweekly for 31 days. Mice alive on day 31 with no parasites found in any blood films were considered protected.

5. Assessment of Causal Prophylactic/Radical Curative Activities in Rhesus Monkeys. The causal prophylactic and radical curative antimalarial activity of the new derivatives **8a** and **8h** were assessed in a *P. cynomolgi* sporozoites challenged Rhesus monkey model. Detailed procedure of sporozoites harvest and drug tests are described in the previous reports.[16−21] The results are shown in Tables 2 and 3. The protocol for assessing causal prophylactic activity of test compounds involved 3 consecutive day treatment of Rhesus monkeys by oral administration, one day before the inoculation of sporozoites, on the day the sporozoites inoculated and a day after the inoculation. Compound **8a** was evaluated side by side with 3 clinical drugs, primaquine, tafenoquine, and Malarone (a fixed combination of atovaquone (10 mg) and proguanil (4 mg)) in the same experiment. Assessment of radical curative activity of the test compounds were carried out using the monkeys that developed parasitemia during the causal prophylactic

Table 2. Causal Prophylactic Activity in Rhesus Monkey Model

group[a]	drug	dose[b] (mg/kg/day × 3)	vehicle	route	first patency day after last treatment	delay patency (days)
1	vehicle	N/A	HECT	po	8	valid control
					8	valid control
2	PQ	1.78	MC	po	13	5
					13	5
3	TQ	0.316	MC	po	11	3
					10	2
4	Malarone	14	HECT	po	11	3
					12	4
5	**8a**	50	HECT	po	19	11
					17	9

[a] Two monkeys per group. [b] Drug was administered on the day before (−1), the day (0), and the day after (+1) sporozoites inoculation; Malarone: a fixed combination of atovaquone (10 mg) plus proguanil (4 mg).

Table 3. Radical Curative Activity in Rhesus Monkey Model

group[a]	test compd drug 1	test compd drug 2	drug 2 dose (mg/kg/day)	drug 2 (route)	relapse result[b] (days)
1	chloroquine 10 mg/kg/day, po for 7 days	none			11
					12
2	chloroquine 10 mg/kg/day, po for 7 days	**8a**	30 × 3 days	im	radical cure (>100)
					74
3	chloroquine 10 mg/kg/day, po for 7 days	**8a**	30 × 7 days	po	81
					32
4	chloroquine 10 mg/kg/day, po for 7 days	**8h**	30 × 7 days	po	26
					21
5	chloroquine 10 mg/kg/day, po for 7 days	PQ	1.78 × 7 days	po	radical cure (>100)
					radical cure (>100)

[a] Two monkeys per group. [b] Number of days relapse occurred after the last day of treatment.

experiments when the test compounds showed no or weak activity. Monkeys were treated with chloroquine (10 mg/kg/day) by oral for 7 consecutive days to clear the blood stage parasites. Relapse occurred after 11−12 days. Test compounds were administered by im or oral for 3 or 7 consecutive days, respectively, after the parasitemia level reached 5000 parasites/mm³. Chloroquine at 10 mg/kg/day × 7 days eliminated 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 were 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 was expected in the control group. Relapse in the treated group indicated failure of the test compounds. Monkeys that showed no relapse after 100 days were considered radically cured. Relapses of the control monkeys were treated with chloroquine once daily for 7 days and observed for the second relapse. Relapse in experimental animals and the second relapse of the control monkeys were treated with the standard 7-day oral CQ and primaquine (1.78 mg base/kg). After standard treatment, blood smears daily for 4 consecutive days and 2 times weekly for 2 weeks were monitored. The results were shown in Table 3.

■ RESULTS AND DISCUSSION

All new compounds were assessed for metabolic stability in human and mouse microsomal preparations with some analogues also tested in Rhesus microsomes. Majority of the new imidazole analogues showed poor metabolic stability in mice microsomal preparations, with $t_{1/2} < 60$ min, but are much more stable in the human microsomal preparation. It is to be noted that the rate of drug metabolism in Rhesus microsome preparations is faster than or about equal to that in mice. This is in sharp contrast to the IZ derivatives, such as compounds **1**, **3**, and **5**, which are metabolically stable in both human and mouse. As was observed with IZ derivatives, the new compounds (**8a−m** and **16a−h**) also showed weak to moderate in vitro antimalarial activities in three clones of *P. falciparum* (D6, W2, and TM91C235) with IC_{50} in the range of 0.3 to >5 μg/mL, with the exception of **8g** (0.3−0.5 μg/mL) and imidazoline analogues **9a−e** (0.2−1.4 μg/mL). Although moderate in vitro activity against *P. falciparum* was observed, compounds **9b−e** also caused toxic deaths in the treated mice.

Among the new compounds tested, only compounds **8a**, **8h**, **9a**, **16a**, and **16e** protected the mice infected with sporozoites of *P. berghei*, at 160 and 320 mg/kg/day by oral dosing. The other compounds, although inactive, showed some degree of growth inhibitory activity against liver stage parasite in the in vivo imaging system (IVIS) test during the first 3 days after the mice were infected with luciferase-labeled sporozoites.[32,33] The results suggest that the imidazole analogues have much smaller bulk tolerance than IZ analogues on the size of substituents R_1 and R_2. For example, the causal prophylactic activity of compounds **8b−f**

with bulky substituents, *t*-butyl or $-CH_2C(CH_3)_3$, at either R_1 or R_2, decrease considerably or totally devoid activity in mice tests. The high efficacy in mice test and poor metabolic stability of **8a** and **8h** plus the unusual SAR results among the tested compounds in Table 1 led us to investigate the metabolite profile of the two active compounds.

Compound **8a** produced a host of metabolites in mouse liver microsome preparations, as well as in blood and in liver homogenate of the treated mice. The mass of major metabolites in LC-mass spectra are m/z 370, 386, and 384 $[M + 1]^+$, which suggest the parent drug **8a** (m/z 354) was metabolized to either 4- or 5-hydroxyl-imidazole derivative (**A** or **A′**), which tautomerized to compound **B** or **B′** (m/z 370) as proposed in Scheme 5.

Scheme 5. Metabolic Pathway of Compound 8a
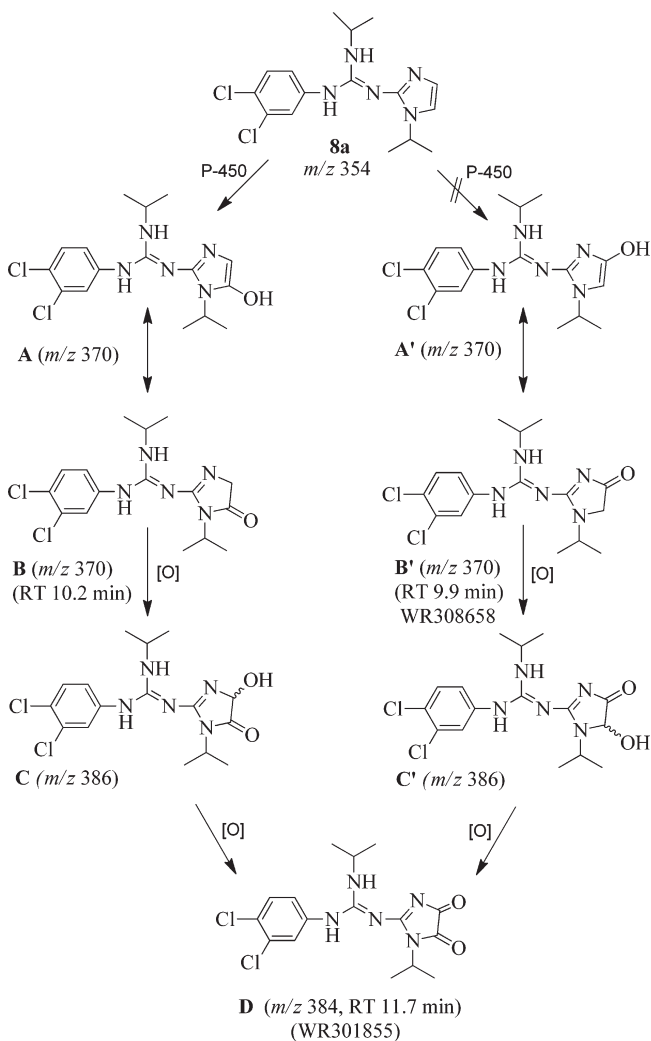
Compound **B** is an unknown compound, but compound **B′** was reported earlier from the authors′ laboratory.[34] Hydroxylation of **B** or **B′** led to formation of **C** or **C′** (m/z 386), which was further oxidized to the stable and known compound WR301855 (**D**, m/z 384). Spiking with authentic sample, metabolite m/z 370 has a different retention time (RT) and ms fragmentation pattern from the known compound **B′**. The RT of metabolite m/z 370 is 10.2 min vs 9.9 min for standard **B′**. Further, the MS^2 spectra of the m/z 370 metabolite in the PK samples consistently show a peak at m/z 328, which is not present in the standard **B′**. The peak m/z 328 $[M^+ - 42]$ is the result of losing a $CH_3CH{=}CH_2$ (m/z 42) fragment from the metabolite m/z 370, a fragmentation pattern that can only occur with compound **B**, but not **B′**, as shown in Scheme 6. Thus, the primary hydroxylation metabolite of **8a** is compound **A**, not **A′**, and the secondary hydroxylation metabolite m/z 386 is compound **C**, not **C′**. Finally, the metabolite m/z 384 has the same m/z, RT, and MS fragmentation pattern as that of the known compound **D** (WR301855).[21] Spiking technique with standard sample further confirmed the structure of metabolite m/z 384 as **D** and established the metabolic pathway of compound **8a** to **D** shown in Scheme 5. As expected, the metabolic pathway of **8h** follows the same pattern as **8a**.

Realizing the stepwise metabolic transformation of compound **8a** to active metabolite **D** (m/z 384) helps to explain the unusual SAR results in Table 1. All compounds **8a**−**m** are metabolically unstable in mouse microsomal preparations, yet only **8a** and **8h** with substituent R_1 = isopropyl are highly active in mice infected with sporozoites. Compounds with bulky substituent such as $-C(CH_3)_3$ or $-CH_2C(CH_3)_3$ at either R_1 (**8d**−**f**, **8i**−**k**), or R_2 (**8b**−**c**) or both (**8d**), are inactive or activity drastically decreased. The plausible explanation of the observation is that compound **8a** acts as a prodrug of metabolite **D**, which was found highly active in causal prophylactic and radical curative tests in Rhesus by im injection.[21] If the contention is correct, the rate of primary hydroxylation of parent compound and/or the secondary hydroxylation of metabolite **B** to **C** constitute the rate limiting step for the generation of active metabolite **D**. Placement of a bulky group at R_1 or R_2 results in steric hindrance to the enzymatic hydroxylation at the 4- or 5-position of the imidazole ring. Consequently, generation of metabolites **B**, **C**, or **D** of analogues with bulky substituent at R_1 or R_2 will be substantially depressed and the antimalarial activity gravely affected.

In a combined causal prophylactic/radical curative Rhesus model, **8a** delayed the patency of treated monkeys for 9−11 days at oral dose of 50 mg/kg/day × 3. In the same test, the first line clinical drugs, primaquine, tafenoquine and Malarone, delayed the patency of treated Rhesus for only 2−5 days at human equivalent dose of 1.78, 0.32, and 14 mg/kg/day × 3, respectively (Table 2). In radical curative test, **8a** cured one monkey and delayed relapse of another for 74 days at 30 mg/kg/day × 7

Scheme 6. MS^2 Fragmentation Pattern of Metabolite B
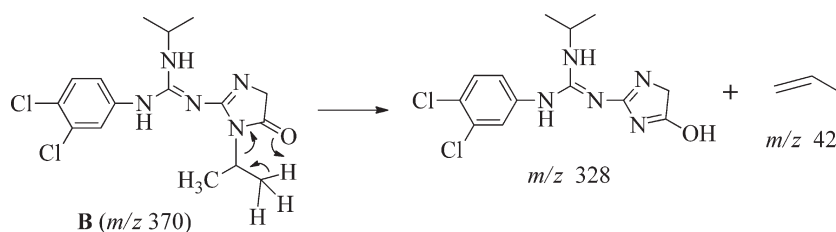
by im, plus 10 mg/kg of chloroquine by oral. By oral administration, **8a** also showed promising activity at the same dose, delayed relapse 81 days for one monkey and 32 days for the other. Control monkeys treated with chloroquine alone relapsed 11−12 days after parasite free. In contrast, compound **8h** which showed superior activity to **8a** in mouse test delayed the relapse of treated monkeys for only 21−26 days at 30 mg/kg/day × 7 by oral (Table 3). The efficacy discrepancy of **8h** observed in mouse and Rhesus tests can be related to the species difference in metabolic stability. The half-life ($t_{1/2}$) of **8h** in mouse is only 27.7 min vs >60 min in Rhesus.

CONCLUSION

Previous studies of guanidyl-imidazolidinedione (IZ) analogues (see refs 17−20) indicated that compounds based on modification of the lead molecule, compound **1**, showed moderate to good causal prophylactic and curative activity in Rhesus only by im, but not by oral. In this study, observation of better causal prophylactic activity of **8a** than primaquine, tafenoquine and Malarone in the same Rhesus test is very encouraging. This is the first guanidylimidazole/guanidylimidazoline derivatives which showed both significant causal prophylactic and radical curative activities in monkeys by oral administration. Finally, the efficacy and metabolite profile data strongly suggest that compound **8a** and its analogues may act as a prodrug of the corresponding IZ antimalarials reported earlier from this laboratory.

ASSOCIATED CONTENT

ⓈSupporting Information. Elemental analysis and X-ray crystallography results. This material is available free of charge via the Internet at http://pubs.acs.org.

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ACKNOWLEDGMENT

ABBREVIATIONS USED

PQ, Primaquine; TQ, tafenoquine; DP, Delayed Parasitemia Patency; IZ, guanidylimidazolinedione derivatives; G6PD, glucose-6-phosphate dehydrogenase

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