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Synthesis and Antiprotozoal Activity of Cationic 1,4-Diphenyl-1*H*-1,2,3-Triazoles

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Abstract

Novel dicationic triazoles **1**–**60** were synthesized by the Pinner method from the corresponding dinitriles, prepared via the copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC). The type and the placement of cationic moieties as well as the nature of aromatic substituents influenced in vitro antiprotozoal activities of compounds **1**–**60** against *Trypanosoma brucei rhodesiense*, *Plasmodium falciparum*, and *Leishmania donovani* and their cytotoxicity for mammalian cells. Eight congeners displayed antitrypanosomal IC_{50} values below 10 nM. Thirty-nine dications were more potent against *P. falciparum* than pentamidine (IC_{50} = 58 nM) and eight analogues were more active than artemisinin (IC_{50} = 6 nM). Diimidazoline **60** exhibited antiplasmodial IC_{50} value of 0.6 nM. Seven congeners administered at 4 × 5 mg/kg by the intraperitoneal route cured at least three out of four animals in the acute mouse model of African trypanosomiasis. At 4 × 1 mg/kg, diamidine **46** displayed better antitrypanosomal efficacy than melarsoprol, curing all infected mice.

Introduction

In recent years, the number of treatment failures associated with the development of drug resistant parasites for many infectious diseases, such as malaria,[1-4] human African trypanosomiasis[5-7] (HATa or sleeping sickness), and leishmaniasis[8-11] has increased with an alarming rate. The majority of people suffering from these infections live in the poorest regions. The need to replace inexpensive commonly used medications that are loosing efficacy due to the development of parasite resistance with pricier alternatives, or to rely on drug combinations, increases the economic burden on the affected nations. In the case of malaria, the structural and mechanistic resemblances of the existing treatments escalate the risk of cross-resistance and potential failures for newly introduced drug candidates. Current therapies approved for use against HAT (which is fatal without treatment) and leishmaniasis require a long course of parenteral administration and suffer from unacceptable toxicity and prolonged dosing.[6, 7, 12] While new medications for visceral leishmaniasis have recently become available,[13, 14] the high cost of treatment limits their broad application in developing countries. Therefore, there is an urgent need for safer and more affordable therapies that are effective against these re-emerging infections.

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[a]Abbreviations: HAT, human African trypanosomiasis; CuAAC, copper(I)-catalyzed azide-alkyne cycloaddition; PMD, pentamidine; MLSP, melarsoprol; CQ, chloroquine; ATMS, artemisinin; PPT, podophyllotoxin.

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Although aromatic diamidines have been long known to possess a wide range of antimicrobial activities,[15-32] only 1,5-bis(4-amidinophenoxy)pentane (pentamidine) (Figure 1) has been widely used to treat humans. The drug has found practical applications against early stage *Trypanosoma brucei gambiense* HAT,[6, 7, 33] antimony-resistant leishmaniasis,[12, 34] and *Pneumocystis jiroveci* (formerly *P. carinii*) pneumonia.[35-37] Additionally, pentamidine demonstrates some antimalarial potency,[23, 38, 39] although it was never approved to treat the disease. Because both cationic moieties in pentamidine are ionized at physiological pH, the drug has low oral activity due to its inability to pass through cellular membranes. This reduces the practicality of pentamidine treatments in remote regions where oral administration of medications would present the most sensible option.

Recently, an orally active prodrug 2,5-bis(4-methoxyamidinophenyl)furan[40] (pafuramidine) (Figure 1) of 2,5-bis(4-amidinophenyl)furan[20] (furamidine) reached Phase III clinical trials against HAT and *P. jiroveci*, although newly recognized possibilities of hepatic and renal toxicity of furamidine in humans are likely to preclude its further development. In a search for novel molecules with improved antiprotozoal activity and reduced toxicity a large number of furamidine-related analogues possessing various aromatic linkers have been synthesized.[19, 21, 29, 41-49] Previously, we described excellent antitrypanosomal and antiplasmodial activities of select cationic diphenyl isoxazoles[50] and 2-phenyl benzofurans.[51] Lately, the 1,2,3-triazole fragment has attracted our attention as a suitable isosteric replacement for the central 5-membered ring because many compounds possessing 1,2,3-triazole fragment exhibit useful biological properties[52-63] and because 1,4-diphenyl 1,2,3-triazoles would retain strong geometrical resemblance to furamidine.

The chemistry of 1,2,3-triazoles has been known for more than a century (for a recent review see Ref.[64]). Among several possible ways to construct the 5-membered triazole ring, the most suitable for us was the Huisgen 1,3-dipolar cycloaddition[65] of organic azides to terminal alkynes. This method, which normally required prolonged usage of elevated temperatures and usually afforded mixtures of 1,4- and 1,5-disubstituted 1,2,3-triazoles,[66, 67] has been recently revitalized by the discovery that Cu(I) salts facilitate the addition of azides to alkynes at ambient temperature affording exclusively 1,4-disubstituted isomers.[68, 69] Because of its high regioselectivity, mild reaction conditions, and excellent yields of desired products, the copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC) has found multiple applications in material science, bioconjugate chemistry, and drug discovery.[70-76]

In our search for novel safer and more potent antiprotozoal drug candidates, we herein report the synthesis of novel cationic 1,4-diphenyl-1,2,3-triazoles **1**–**60** utilizing the CuAAC methodology. Compounds **1**–**60** were evaluated in vitro for antiprotozoal potency versus *T. brucei rhodesiense* (STIB900), chloroquine resistant *Plasmodium falciparum* (K1), axenic amastigotes of *Leishmania donovani* (MHOM/SD/62/1S–$CL2_D$), and for cytotoxicity against rat myoblast cells (L6). Dications exhibiting high antitrypanosomal activities in vitro were screened in vivo in the STIB900 acute mouse model of African trypanosomiasis.

Chemistry

3-Azidobenzonitrile[77] (**61**) and 4-azidobenzonitrile[77, 78] (**62**) were obtained by diazotization/azidation of the corresponding commercially available aminobenzonitriles following the published procedure.[79, 80] Synthesis of novel phenyl azides **63**–**65** is depicted in Scheme 1. 4-Methoxy-3-nitrobenzonitrile (**67**), prepared by O-methylation of commercially available 4-hydroxy-3-nitrobenzonitrile (**66**) with MeI and K_2CO_3 in DMF, underwent catalytic hydrogenation to give 3-amino-4-methoxybenzonitrile (**68**). Compound **68** was diazotized with sodium nitrite in diluted hydrochloric acid at 0–5 °C followed by azidation of the resulting diazonium chloride with sodium azide to afford 3-azido-4-

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methoxybenzonitrile (**63**) in 64% yield over the three steps. 4-Bromo-*o*-anisidine (**70**) was synthesized in 50% yield by bromination of *o*-anisidine (**69**) with bromine in glacial acetic acid as previously described.[81] Compound **70** was reacted with CuCN in DMF–pyridine (5:1) mixture to give 4-amino-3-methoxybenzonitrile[82] (**71**) in 54% yield. Treatment of **71** with sodium nitrite in hydrochloric acid at 0–5 °C followed by reaction with sodium azide afforded 4-azido-3-methoxybenzonitrile (**64**) in 94% yield. 3-Azido-4-hydroxybenzonitrile (**65**) was obtained in 55% overall yield from **66** by catalytic hydrogenation over 10% Pd/C followed by the diazotization/azidation of the resulting 3-amino-4-hydroxybenzonitrile (**72**). Ethynyl benzonitriles **73**–**76** were synthesized as reported earlier.[50]

1,4-Dicyanophenyl-1*H*-1,2,3-triazoles **77**–**89** and **93** were prepared by the copper(I)-catalyzed 1,3-dipolar cycloaddition[70, 74] of 3- or 4-azidobenzonitriles **61**–**65** to ethynyl benzonitriles **73**–**76** in aqueous *t*-BuOH or DMSO following the published protocol (Scheme 2).[69] Hydroxy substituted dinitriles **90**, **92**, **94**, and **96** were synthesized from the corresponding methoxy substituted congeners **82**, **84**, **86**, and **88** in 89–99% yields by treatment with BBr_3 in CH_2Cl_2. Attempts to employ this method for demethylation of methoxy derivatives **83** and **87** were unsuccessful. Consequently, compounds **91** and **95** were obtained in 89% yields by treatment of the dinitriles **83** and **87** with melted pyridine hydrochloride at 150–160 °C following the previously reported procedure.[51] Dicationic 1,4-diphenyl-1*H*-1,2,3-triazoles **1**–**60** (Tables 1-4) were synthesized using the modified Pinner method[83] (Scheme 3). Thus, dinitriles **77**–**96** were transformed to imidate esters, which reacted with ethanolic solutions of ammonia, isopropylamine, or ethylenediamine at ambient temperature followed by treatment with aqueous HCl to afford congeners **1**–**60** as dihydrochloride salts.

Results and Discussion

In our recent study of 1,4-diphenyl isoxazoles, we found that several methoxy substituted dications were more active against *T. brucei rhodesiense* and *P. falciparum* compared to their chloro and nitro substituted analogues.[50] Similarly, the introduction of methoxy and hydroxy substituents improved antitrypanosomal and antiplasmodial activities and reduced cytotoxicities of select 2-phenyl benzofurans.[51] Here, we investigate how the substitution on amidine groups, varying the position of the cationic moieties, and the attachment of methoxy and hydroxy groups to the aromatic rings influences antiprotozoal properties of novel cationic diphenyl triazoles **1**–**60**. The results of the in vitro testing of the compounds **1**–**60** against the bloodstream form of *T. brucei rhodesiense* trypomastigotes (STIB900), chloroquine resistant *P. falciparum* (K1), axenic amastigotes of *L. donovani* (MHOM/SD/62/1S-$CL2_D$), and the assessment of their cytotoxicity against rat myoblast cells (L6) are summarized in Tables 1-4. For comparison, we included the activities of pentamidine, melarsoprol (*T. brucei rhodesiense*), chloroquine and artemisinin (*P. falciparum*), and podophyllotoxin (L6). To differentiate between cytotoxicity for parasite and mammalian cells, three in vitro selectivity indeces[84] were calculated as follows: antitrypanosomal selectivity index SI_T, expressed as the ratio [IC_{50} (L6) / IC_{50} (*T. brucei rhodesiense*)], antiplasmodial selectivity index SI_P, expressed as the ratio [IC_{50} (L6) / IC_{50} (*P. falciparum*)], and antileishmanial selectivity index SI_L, expressed as the ratio [IC_{50} (L6) / IC_{50} (*L. donovani*)]. The results of the in vivo screening of select dications in the STIB900 acute mouse model of trypanosomiasis are presented in Table 5.

Structure–Activity Relationship

Cytotoxicity Study—Except for diamidines **1**, **16**, **31**, **37**, **46**, **52**, and **58**, bis(*N*-isopropyl)amidines **5** and **32** and diimidazolines **18**, **33**, **38**, and **54**, all tested triazoles exhibited no cytotoxicity for L6 mammalian cells at the maximum dose of 90 μg/mL. Out of

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thirteen congeners active in the L6 assay, four compounds (**1**, **5**, **16**, and **18**) possessed one cationic moiety in the 5′-position and nine were 4′,5 - (**31**–**33**, **37**) and 4,4′-disubstituted analogues (**46**, **48**, **52**, **54**, **58**). Bis(*N*-isopropyl)amidines and diimidazolines without methoxy and hydroxy substituents on aromatic rings were less cytotoxic for mammalian cells than the corresponding diamidines. Placement of one cationic group in the 4-position increased cytotoxicities of 4,5′- and 4,4′-disubstituted diamidines **16** and **46** compared to the 5,5′-disubstituted analogue **1** nearly 25-fold. Although 4′,5-disubstituted congener **31** also displayed increased cytotoxicity with respect to diamidine **1**, it was less cytotoxic than 4,5′- and 4′,4-disubstituted isomers **16** and **46**. Apart from 4-substituted diamidines **16** and **46**, all tested congeners **1**–**60** exhibited lower cytotoxicities than pentamidine.

In Vitro Antitrypanosomal Activity

All compounds **1**–**60** were active in vitro against *T. brucei rhodesiense* showing antitrypanosomal IC_{50} values ranging from 4 nM to 102 μM. Eight diamidines (**16**, **19**, **22**, **28**, **31**, **37**, **43**, and **46**) and diimidazoline **9** displayed antitrypanosomal IC_{50} values comparable to that of melarsoprol (IC_{50} = 4 nM) and pentamidine (IC_{50} = 3 nM). Diamidine **46** bearing cationic groups in the 4,4′-positions was the most potent compounds in the series with antitrypanosomal IC_{50} value of 4 nM.

The N-substitution on amidine groups has been shown to reduce antitrypanosomal properties of aromatic diamidines. In this study, unsubstituted diamidines, except congeners **7** and **10**, also exhibited higher in vitro activities against *T. brucei rhodesiense* than the corresponding bis(*N*-isopropyl)amidines and diimidazolines, corroborating previously published results.[23, 50, 85-89] For instance, 5,5′-disubstituted bis(*N*-isopropyl)amidine **2** and diimidazoline **3** were nearly 170-fold less active against *T. brucei rhodesiense* compared to diamidine **1** (Table 1). This loss of antitrypanosomal activity was further enhanced by the introduction of the methoxy and hydroxy groups on aromatic rings. For example, antitrypanosomal potencies of 4,5′-disubstituted bis(*N*-isopropyl)amidine **17** and diimidazoline **18** decreased 67- and 25-fold compared to that of diamidine **16**. However, their 2-methoxy substituted analogues **20** and **21** were 178- and 170-fold less active against *T. brucei rhodesiense* than the corresponding congener **19** (Table 2). Similarly, in the series of the 4′,5-disubstituted triazoles (Table 3), activities of bis(*N*-isopropyl)amidine **32** and diimidazoline **33** for the pathogen decreased 77-fold compared to diamidine **31**. At the same time, dications **38** and **39** bearing the methoxy group in the 2′-position were 141- and 804-fold less active against *T. brucei rhodesiense* than diamidine **37**. This pattern continued among the 4,4′-disubstituted congeners (Table 4). Overall, antitrypanosomal potency of dications without methoxy and hydroxy groups on aromatic rings decreased in the order Am > Im > *i*-PrAm, whereas compounds possessing these substituents displayed no apparent correlation between the nature of cationic moieties and activities versus *T. brucei rhodesiense*.

Placement of cationic groups on aromatic rings affected the antitrypanosomal properties of triazoles **1**–**60**. Among the dications lacking methoxy and hydroxy substituents, 4,4′-disubstituted analogues **46**–**48** were more active against *T. brucei rhodesiense* compared to isomers possessing cationic moieties in the 5- or 5′-positions. Alternatively, 4,4′-disubstituted diamidines **49**, **52**, and **58** bearing methoxy or hydroxy groups exhibited lower antitrypanosomal activities than the corresponding 4,5′- and 4′,5-disubstituted analogues **19**, **22**, and **28** and **34**, **37**, and **43**, respectively. In the case of methoxy and hydroxy substituted dications, all 5,5′-disubstituted congeners, except 2′-methoxy diimidazoline **9** and 2-hydroxy bis(*N*-isopropyl)amidine **11**, were less active against *T. brucei rhodesiense* than their counterparts possessing at least one of the cationic moieties in the 4- or 4′-positions. Thus, among the methoxy and hydroxy substituted analogues, 4,5′-disubtituted diamidines as well

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as 4,4′-substituted bis(*N*-isopropyl)amidines and diimidazolines were the most potent against *T. brucei rhodesiense*.

The effect of the methoxy and hydroxy substitution on antitrypanosomal activities of dications **1–60** varied based on the type and the position of cationic moieties and the nature of aromatic substituents. For example, in the series of the 5,5′-disubstituted analogues **1**–**15** (Table 1), the attachment of the methoxy group to the 2-position of diamidine **1** and diimidazoline **3** improved activities of the resulting 2-methoxy analogues **4** and **6** against *T. brucei rhodesiense* with respect to the parent compounds. At the same time, 2-hydroxy substituted congeners **10** and **12** displayed antitrypanosomal potencies 600- and 7-fold lower relative to dications **1** and **3**. 2-Methoxy substituted diamidine **4** and diimidazoline **6** were 1000- and 13-fold as active against *T. brucei rhodesiense* as their 2-*O*-demethylated analogues **10** and **12**, while both bis(*N*-isopropyl)amidines **5** and isomer **11**, bearing methoxy and hydroxy substituents in the 2-position, exhibited antitrypanosomal potencies comparable to that of congener **2**. Similarly, activities of 2′-substituted methoxy and hydroxy diamidines **7** and **13** against *T. brucei rhodesiense* decreased 20- and 2-fold relative to the unsubstituted congener **1**, while 2′-methoxy and 2′-hydroxy bis(*N*-isopropyl)amidines **8** and **14** were more potent than analogue **2**. In the case of diimidazolines **9** and **15**, the attachment of the 2′-hydroxy group only marginally improved antitrypanosomal activity of congener **15**, while 2′-methoxy substituted diimidazoline **9** was nearly 2350-times as active against the pathogen as unsubstituted analogue **3**.

In the series of the 4,5′-disubstituted congeners **16**–**30** (Table 2), diamidines **19**, **22**, and **28** possessing 2- and 2′-methoxy as well as 2′-hydroxy groups displayed antitrypanosomal potencies comparable to that of the unsubstituted dication **16**. At the same time, the introduction of the 2-hydroxy substituent reduced the activity of the resulting congener **25** against the pathogen nearly 22-fold relative to diamidine **16**. 2-Substituted bis(*N*-isopropyl)amidines and diimidazolines **20**, **21**, **26**, and **27** were less active versus *T. brucei rhodesiense* than dications **17** and **18**, while the attachment of the methoxy or hydroxy groups to the 2′-position increased antitrypanosomal activities of congeners **23**, **24**, **29**, and **30**.

Antitrypanosomal properties of the 4′,5-disubstituted dications **31**–**45** (Table 3) varied depending on the nature of cationic moieties and aromatic substituents. For example, while the placement of the methoxy and hydroxy groups in the 2-position reduced activities of diamidines **34** and **40** against *T. brucei rhodesiense* relative to congener **31**, 2′-substituted diamidines **37** and **43** displayed potencies comparable to that of the unsubstituted analogue **31**. Bis(*N*-isopropyl)amidines and diimidazolines, however, demonstrated different trends. For instance, antitrypanosomal activities of 2-methoxy substituted bis(*N*-isopropyl)amidine **35** and diimidazoline **36** improved compared to congeners **32** and **33**, while the placement of the hydroxy group in the same position reduced the activity of bis(*N*-isopropyl)amidine **41** and diimidazoline **42** against the pathogen 33- and 43-fold, respectively. On the other hand, antitrypanosomal activities of 2′-methoxy substituted bis(*N*-isopropyl)amidine **38** and diimidazoline **39** decreased relative to dications **32** and **33**. At the same time, 2′-hydroxy substituted congener **44** was more potent against *T. brucei rhodesiense* than bis(*N*-isopropyl)amidine **32**, while dication **45** was less active than diimidazoline **33**.

Except for 2′-hydroxy substituted bis(*N*-isopropyl)amidine **59** and diimidazoline **60**, the introduction of the methoxy and hydroxy groups on aromatic rings did not improve activities of 4,4′-disubstituted congeners **49**–**60** against *T. brucei rhodesiense* relative to analogues **46**–**48** (Table 4). For example, placement of the hydroxy group in the 2-position reduced the antitrypanosomal activity of diamidine **55** 125-fold compared to dication **46**. Bis(*N*-isopropyl)amidine **59** and diimidazoline **60** were more potent than the corresponding

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unsubstituted analogues **47**, **48** and dications **53**, **54**, possessing the methoxy group in the 2′-position.

Dications **1**–**60** displayed in vitro antitrypanosomal selectivity indices SI_T, varied from 2 to 36500. All diamidines except congeners **7**, **10**, and **58** possessed higher selectivity indices than bis(*N*-isopropyl)amidines and diimidazolines, which correlate with our earlier findings for 2-phenyl benzofurans.[51] Six dications (**9**, **19**, **22**, **28**, **37**, and **43**) exhibited antitrypanosomal selectivity indices greater than that of pentamidine (SI_T = 15533), while 4,5′-disubstituted diamidine **28** had the highest selectivity index for *T. brucei rhodesiense* in the series (SI_T = 36500).

In Vitro Antiplasmodial Activity

All tested dications **1**–**60** exhibited in vitro antiplasmodial activities with IC_{50} values ranging from 0.6 nM to 8.93 μM. Thirty-nine dications, including sixteen diamidines (**1**, **4**, **7**, **13**, **16**, **19**, **22**, **28**, **31**, **34**, **37 43**, **46**, **49**, **52**, and **58**), eleven bis(N-isopropyl)amidines (**17**, **20**, **23**, **29**, **32**, **38**, **44**, **47**, **50**, **53**, and **59**), and twelve diimidazolines (**6**, **9**, **15**, **24**, **30**, **36**, **45**, **48**, **51**, **54**, **57**, and **60**), were more potent in vitro against *P. falciparum* than pentamidine (IC_{50} = 58 nM). Eight analogues (**16**, **31**, **46**–**48**, **52**, **58**, and **60**) displayed better antiplasmodial activities in vitro than artemisinin (IC_{50} = 6 nM). 4,4′-Disubstituted diimidazoline **60** showing antiplasmodial IC_{50} value of 0.6 nM was the most potent compounds in the series against *P. falciparum*.

In this study, the influence of the N-alkylation of cationic moieties on antiplasmodial properties of tested dications **1**–**60** was not conclusive. Thus, in the series of congeners lacking methoxy and hydroxy substituents on aromatic rings, diamidines were generally more active against *P. falciparum* than bis(*N*-isopropyl)amidines and diimidazolines. At the same time, among the methoxy and hydroxy substituted analogues, we found no apparent correlation between the nature of cationic moieties and antiplasmodial activities.

Placement of cationic moieties in the 4- or 4′-positions increased in vitro antiplasmodial activities of tested congeners. For example, all 4,5′-disubstituted congeners **16**–**30**, except for 2-methoxy substituted diimidazoline **21** and 2-hydroxy substituted bis(*N*-isopropyl)amidine **26**, displayed higher antiplasmodial activities compared to isomers **1**–**15** bearing cationic moieties in the 5,5′-positions. Similarly, congeners **31**–**45** were more active against *P. falciparum* than 5,5′-disubstituted isomers **1**–**15** except for bis(*N*-isopropyl)amidines **35** and **41**, possessing the methoxy and hydroxy groups in the 2-position, and 2′-methoxy substituted diimidazoline **39** and 2-hydroxy substituted isomer **42**. 4,4′-Disubstituted dications **46**–**60** were more active against *P. falciparum* than dications **1**–**45** bearing at least one cationic moiety in the 5- or 5′-positions, except for 2′-methoxy substituted diimidazoline **54** and 2-hydroxy substituted bis(*N*-isopropyl)amidine **56** that were less potent than the corresponding 5,5′-disubstituted analogue **11** and 4,5′-disubstituted isomer **24**, respectively. All 4,4′-disubstituted congeners, excluding analogues **55**–**57** possessing the hydroxy group in the 2-position, exhibited antiplasmodial IC_{50} values below 20 nM.

The effect of the methoxy and hydroxy substitution on antiplasmodial activities of dications **1–60** varied depending on the nature and the position of cationic moieties. For example, in the series of 5,5′-disubstituted analogues (Table 1), 2- and 2′-methoxy substituted diamidines **4** and **7** as well as the corresponding bis(*N*-isopropyl)amidines **5** and **8** exhibited in vitro activities versus *P. falciparum* comparable to those of unsubstituted dications **1** and **2**. At the same time, activities of 2′-hydroxy substituted diamidine **13** and bis(*N*-isopropyl)amidine **14** against the parasite decreased slightly compared to analogues **1** and **2**, while placement of the hydroxy group in the 2-position significantly affected antiplasmodial

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activities of dications **10**–**12**. Thus, 2-hydroxy substituted diamidine **10** was 425- and 320-fold less potent against *P. falciparum* compared to unsubstituted dication **1** and its 2-methoxy substituted analogue **4**. Similarly, bis(*N*-isopropyl)amidine **11** exhibited 7- and 6-fold lower antiplasmodial activity relative to congeners **2** and **5**. On the other hand, 2- and 2′-methoxy substituted diimidazolines **6** and **9** and 2′-hydroxy substituted analogue **15** were 12-, 26-, and 7-fold more active against *P. falciparum* than unsubstituted diimidazoline **3**, while dication **12** possessing hydroxy group in the 2-position displayed antiplasmodial activity comparable to that of **3**.

Among the 4,5′-disubstituted analogues **16**–**30** (Table 2), methoxy and hydroxy substituted diamidines **19**, **22**, **25**, and **28** were less active in vitro against *P. falciparum* than unsubstituted analogue **16**. In the case of bis(*N*-isopropyl)amidines, placement of the methoxy or hydroxy group in the 2-position reduced antiplasmodial activities of dications **20** and **26** compared to unsubstituted isomer **17**, while 2′-methoxy- and hydroxy substituted congeners **23** and **29** exhibited higher activities against the parasite. At the same time, all 2- and 2′-substituted diimidazolines except for congener **27**, possessing the hydroxy group in the 2-position, were more active against *P. falciparum* than unsubstituted dication **18**.

In the series of 4′,5-disubstituted congeners **31**–**45** (Table 3), the effect of the methoxy or hydroxy substitution on aromatic rings on antiplasmodial potencies depended on the type of the cationic moieties and the position of aromatic substituents. For example, 2′-methoxy and 2′-hydroxy substituted diamidines **37** and **43** were only marginally less active against *P. falciparum* compared to the unsubstituted analogue **31**, while the antiplasmodial activity of dication **34**, possessing the methoxy group in the 2-position, decreased 4-fold. At the same time, 2-hydroxy substituted diamidine **40** was 308-fold less active versus the parasite than congener **31**. Also, bis(*N*-isopropyl)amidines **38** and **44**, possessing the methoxy and hydroxy groups in the 2′-position, displayed antiplasmodial activities comparable to that of dication **32**. On the other hand, 2-methoxy substituted analogue **35** and 2-hydroxy substituted isomer **41** were 5- and 123-fold less active against *P. falciparum* than unsubstituted bis(*N*-isopropyl)amidine **32**. All methoxy and hydroxy substituted diimidazolines except congener **42**, possessing the hydroxy group in the 2-position, displayed improved antiplasmodial activities compared to the unsubstituted diimidazoline **33**.

Similarly, the antiplasmodial properties of analogues **46**–**60** (Table 4) also depended on the placement of aromatic substituents. Thus, regardless of the nature of the cationic moieties, the attachment of methoxy or hydroxy groups in the 2-position reduced antiplasmodial activities of congeners **49**–**51** and **55**–**57**, respectively, compared to the unsubstituted analogues **46**–**48**. At the same time, 2′-methoxy and 2′-hydroxy substituted diamidines **52** and **58** and bis(*N*-isopropyl)amidines **53** and **59** displayed comparable activities against *P. falciparum* relative to unsubstituted congeners **46** and **47**. The antiplasmodial potency of diimidazoline **54**, possessing the methoxy group in the 2′-position, decreased relative to that of unsubstituted congener **48**, while 2′-hydroxy substituted analogue **60** was 3 times as active against the parasite as diimidazoline **48** and 10 times as potent as artemisinin. Diimidazoline **60** (IC_{50} = 0.6 nM) was the most active compound among all tested congeners **1**–**60** against *P. falciparum*. Compound **60** was tested in vivo in the *P. berghei* mouse model. When administered subcutaneously at 30 mg/kg daily for four days, diimidazoline **60** did not reduce the blood parasite count (data not shown). This result, although discouraging, came at no surprise, because aromatic diamidines do not usually display good activity in this model. For example, orally active pafuramidine dosed at 100 mg twice a day for 5 days has demonstrated 90% efficacy in uncomplicated *P. falciparum* malaria and 80% in *P. vivax* malaria in humans,[90] even though furamidine was inactive against trophozoite-induced *P. berghei* infection in mice.[20, 85]

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Compounds **1**–**60** displayed in vitro antiplasmodial selectivity indices SI_P, varied from 23 to >316667. Among dications with identical substitution pattern on aromatic rings (unsubstituted, methoxy- or hydroxy substituted), the selectivity indices of 5,5′- and 4′,5-disubstituted congeners **1**–**15** and **31**–**45** for *P. falciparum* correlated with their activities against the parasite. Thus, congeners **1**, **4**, **9**, and **13**, showing superior activities among dications possessing the same aromatic substituents, also displayed maximum selectivity indices for *P. falciparum*. The selectivity indices of congeners **16**–**30** varied depending on the nature of cationic moieties and the substitution on the aromatic rings. For example, in the case of unsubstituted or methoxy substituted dications **16**–**24**, diamidines **16**, **19**, and **22** demonstrated maximum antiplasmodial selectivity indices, while among the hydroxy substituted analogues **25**–**30**, selectivity indices of diimidazolines **27** and **30** were the highest. Antiplasmodial selectivity indices of congeners **22**–**24** and **28**–**30** bearing methoxy or hydroxy groups in the 2′-position increased compared to both dications **16**–**18** lacking aromatic substituents and to the corresponding 5,5′-disubstituted analogues **7**–**9** and **13**–**15**. Except for 2-hydroxy substituted congeners **10**–**12** and **40**–**42** and bis(*N*-isopropyl)amidines **26** and **56** also possessing the hydroxy group in the 2-position, dications **1**–**60** exhibited superior antiplasmodial selectivity indices for *P. falciparum* compared to pentamidine. 4,4′-Disubstituted diimidazoline **60** exhibited the highest antiplasmodial selectivity index in the series (SI_P = >316667), which was nearly 400 times greater than that of pentamidine.

In Vitro Antileishmanial Activity

Among the 5,5′-disubstituted triazoles **1**–**15** (Table 1), only diamidine **1** and its 2′-hydroxy substituted isomer **13** as well as diimidazoline **9**, possessing the methoxy group in the 2′-position, were active against *L. donovani*. The substitution on the amidine moieties had a distinct effect on the antileishmanial properties of 4,5′-disubstituted congeners **16**–**30** (Table 2). Only diamidine **16** and the corresponding 2- and 2′-substituted analogues **19** and **22**, as well as dications **28** and **30** bearing the 2′-hydroxy group were active against *L. donovani*. Attachment of the 2′-methoxy substituent improved the antileishmanial potency of the diamidine **22** compared to congener **16**. Antileishmanial potency of congeners **31**–**45** (Table 3) diminished with the substitution on the amidine moieties. Except for diamidines **31**, **34**, **37**, and **43** and bis(*N*-isopropyl)amidines **32** and **38**, the 4′,5-substituted congeners were inactive against *L. donovani*. Antileishmanial activity of the dications **31**–**45** improved compared to their 5,5′-substituted counterparts **1**–**15**. 2′-Hydroxy substituted diamidine **43** was more active than both the unsubstituted dication **31** and its 2′-methoxy analogue **37**. The substitution on amidine groups or on aromatic rings in most cases reduced the antileishmanial potency of congeners **46**–**60** (Table 4). Except for 2′-hydroxy substituted diamidine **58** and diimidazoline **60**, all 4,4′- disubstituted congeners **49**–**60** bearing methoxy or hydroxy groups on aromatic rings were less active against *L. donovani* than unsubstituted dications **46**–**48**. Similar to 4′,5-disubstituted isomers, 2′-hydroxy substituted diamidine **58** was the most active compound among dications **46**–**60**, demonstrating improved antileishmanial potency compared to both unsubstituted dication **46** and 2′-methoxy substituted congener **52**.

In Vivo Antitrypanosomal Activity

Selected 1,4-diphenyl-1*H*-1,2,3-triazoles exhibiting promising in vitro activities against *T. brucei rhodesiense* were evaluated in the STIB900 mouse model of African trypanosomiasis (Table 5). The screening was conducted using intraperitoneal dosing at 5 mg/kg daily for 4 days. Diamidines **22**, **28**, **37**, **43**, **46**, **52**, and **58**, bis(*N*-isopropyl)amidine **59**, and diimidazoline **9** displayed excellent in vivo efficacies curing at least two out of four animals.

With the exception of 2′-hydroxy substituted bis(*N*-isopropyl)amidine **59**, *N*-substitution on the cationic fragments reduced antitrypanosomal efficacies of diphenyl triazoles. For

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example, all tested diimidazolines except 5,5′-disubstituted dication **9**, possessing the methoxy group in the 2′-position, displayed no curative activity at this dosage. Besides, 4,5′-disubstituted bis(*N*-isopropyl)amidine **23** and 4,4′-disubstituted analogue **47** were found to be toxic in the in vivo model despite showing no in vitro toxicity. However, this lack of correlation between the in vitro and the in vivo data is not surprising and quite common. The purpose of the in vitro cytotoxicity assay is to identify and discharge compounds exhibiting antiprotozoal activity due to general cellular toxicity.[84] Because only toxic effects at a single cellular level can be observed in vitro, it is impossible to detect the organ-specific toxicity of the compound in question until in vivo animal tests are conducted.

The position of attachment of cationic moieties influenced the efficacy of tested dications in the acute mouse model of African trypanosomiasis (compare **1**, **16**, **31**, and **46**). For example, 5,5′-disubstituted congener **1** displayed no activity, while diamidines **16** and **31** bearing one cationic group in the 4- or 4′-positions revealed improved antitrypanosomal efficacies, curing one out of four mice. At the same time, 4,4′-disubstituted diamidine **46** provided cures to all infected animals.

The placement of methoxy and hydroxy groups affected antitrypanosomal efficacies of tested analogues depending on the placement of cationic moieties. For example, antitrypanosomal potency of 4,5′- and 4′,5-substituted diamidines **19** and **34** bearing the methoxy group in the 2-position did not improve compared to their unsubstituted counterparts **16** and **31**. On the other hand, 2′-methoxy substituted congeners **22** and **37** and 2′-hydroxy substituted analogues **28** and **43** were more active than diamidines **16** and **31**, lacking aromatic substitution. At the same time, the introduction of the aromatic substituents reduced antitrypanosomal efficacy of 4,4′-disubstituted congeners with respect to diamidine **46**.

Dications **9**, **37**, and **46**, which cured all mice at the 5 mg/kg dosing, were subjected to a dose-response evaluation. In a single-dose regimen at 10 mg/kg, diimidazoline **9** and diamidine **46** cured three out of four animals. When administered at 1 mg/kg daily for 4 days, dications **9** and **37** provided no cures, while congener **46** retained its in vivo efficacy curing all mice. Further reduction of the administered dosage of diamidine **46** to 0.5 mg/kg daily for 4 days afforded no cures but substantially postponed relapse time for infected animals compared to untreated controls. Overall, dication **46** exhibited excellent potency in the STIB900 acute mouse model of African trypanosomiasis. To our knowledge, this is the first aromatic diamidine showing in vivo efficacy superior to that of melarsoprol.

Summary

A series of cationic 1,4-diphenyl-1*H*-1,2,3-triazoles **1**–**60** was synthesized by the Pinner method from the corresponding dinitriles, prepared via the copper(I)-catalyzed azide–alkyne cycloaddition (CuAAC). Dications **1**–**60** were tested in vitro against *T. brucei rhodesiense*, *P. falciparum*, and *L. donovani* as well as for cytotoxicity against mammalian cells. The cytotoxicities of triazoles **1**–**60** were lower compared to that of pentamidine and were not significantly affected by the alkylation on the amidine groups or the substitution on the aromatic rings. However, the placement of the cationic moiety in the 4-position increased the cytotoxicities of diamidines **16** and **46** with respect to the drug. Except for the congeners **7** and **10**, unsubstituted diamidines exhibited higher in vitro activities against *T. brucei rhodesiense*than bis(*N*-isopropyl)amidines and diimidazolines, corroborating previously published results.[50, 85-87, 89] The majority of diamidines also exhibited higher antiplasmodial and antileishmanial activities compared to N-substituted analogues. The position of attachment of cationic groups influenced antiprotozoal properties of triazoles **1**–**60**. For example, placement of the cationic fragments in the 5,5′-position of the aromatic

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rings afforded congeners that were in most cases less potent against *T. brucei rhodesiense*, *P. falciparum* and *L. donovani* than isomers bearing at least one cationic moiety in the 4- or 4′-positions. The introduction of the hydroxy group in the 2-position of the phenyl ring significantly reduced activities of tested congeners against *T. brucei rhodesiense*, *P. falciparum* and *L. donovani*, perhaps due to the formation of the intramolecular hydrogen bond between the hydrogen atom of the 2-hydroxy group and the 2-nitrogen of the triazole ring. Diamidines **28**, **37**, **46**, and **58**, bis(*N*-isopropyl)amidine **59**, and diimidazoline **9** exhibited excellent in vivo efficacies in the acute mouse model of trypanosomiasis curing all infected animals when administered intraperitoneally at 5 mg/kg daily for 4 days. Even at a lower dose of 1 mg/kg diamidine **46** retained its curative potency exhibiting in vivo efficacy superior to that of melarsoprol. Promising antiprotozoal activity of cationic 1,4-diphenyl-1*H*-1,2,3-triazoles **1**–**60** in vitro and excellent efficacy of select congeners in the acute mouse model of African trypanosomiasis warrant further investigation of this class of compounds as potential antitrypanosomal drug candidates.

Experimental Section

Biology

Preparation of Compounds: Compounds were dissolved in 100% dimethylsulfoxide (DMSO) and finally diluted in culture medium prior to the assay. The DMSO concentration never exceeded 1% in the in vitro assays. For in vivo experiments, the compounds were dissolved in DMSO and further diluted with distilled H_2O to a final DMSO concentration of 10% prior to injection into the animals.

In Vitro Cytotoxicity Assay (L6 Rat Myoblast Cells)—IC_{50} values were determined using the Alamar blue assay[91] and were carried out twice independently and in duplicate. Briefly, 4000 L6 cells were seeded in RPMI 1640 medium supplemented with L-glutamine 2 mM, HEPES 5.95 g/L, $NaHCO_3$ 2 g/L, and 10% fetal bovine serum in 96-well microtiter plates. The serial drug dilutions were incubated for 70 h at 37 °C under a humidified 5% CO_2 atmosphere. The viability marker Alamar blue (12.5 mg resazurin (Sigma) dissolved in 100 ml phosphate buffered saline) (10 μL) was then added to each well and the plate was incubated for additional 2–3 h. The plates were read in a Spectramax Gemini XS microplate fluorescence scanner (Molecular Devices) using an excitation wavelength 536 nm and an emission wavelength 588 nm. The IC_{50} values were calculated from the sigmoidal inhibition curves using the SoftmaxPro software.

In Vitro Growth Inhibition Assay of *T. brucei rhodesiense* (STIB900)—IC_{50} values were determined using the Alamar blue assay and were carried out twice independently and in duplicate. Briefly, the compounds were tested in Minimum Essential Medium with Earle's salts, supplemented as previously described[92] with the following modifications: 2-mercaptoethanol 0.2 mM, sodium pyruvate 1 mM, hypoxanthine 0.5 mM, and 15% heat-inactivated horse serum. Serial drug dilutions were prepared in 96-well microtiter plates and each well inoculated with 2000 bloodstream forms and incubated for 70 h at 37 °C under a humidified 5% CO_2 atmosphere. The viability marker Alamar blue (12.5 mg resazurin (Sigma) dissolved in 100 ml phosphate buffered saline) (10 μL) was then added to each well and the plate was incubated for additional 2–6 h. The plates were read in a Spectramax Gemini XS microplate fluorescence scanner (Molecular Devices) using an excitation wavelength of 536 nm and an emission wavelength of 588 nm. The IC_{50} values were calculated from the sigmoidal inhibition curves using the SoftmaxPro software.

In Vitro Growth Inhibition Assay of *P. falciparum*(K1)—The determination of IC_{50} values against erythrocytic stages of *P. falciparum*was carried out twice independently and in duplicate using the [^{3}H]-hypoxanthine incorporation assay.[93, 94] Briefly, the compounds

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were tested in RPMI 1640 medium 10.44 g/L, supplemented with Hepes 5.94 g/L, Albumax II 5 g/L, sodium bicarbonate 2.1 g/L, and neomycin 100 mg/L in 96-well microtiter plates. Infected human red blood cells in medium (hematocrit 1.25%, parasitemia 0.3%) were incubated with the drug dilutions in an atmosphere of 93% N_2, 4% CO_2, 3% O_2 at 37 °C. After 48 h, [^{3}H]-hypoxanthine (0.5 μCi/well) was added and the plates were incubated for additional 24 h under the same conditions. The wells were harvested with a Betaplate cell harvester and transferred on a glass fiber filter. Viability was assessed by measuring the incorporation of [^{3}H]-hypoxanthine by a Betaplate liquid scintillation counter (Wallac, Zurich, Switzerland). The IC_{50} values were calculated from the sigmoidal inhibition curves using MS Excel.

Antileishmanial Assay—Axenic amastigotes of *L. donovani* (WHO designation MHOM/SD/62/1S-CL2$_D$) were adapted from promastigotes and grown in the amastigote medium described previously[95] at 37 °C. In a final volume of 60 μL, 6 × 10^4 parasites were added to each well of a 96-well plate except for negative control wells. Standard and test compounds were added as appropriate using 2-fold dilutions to allow a range of concentrations to be tested. Plates were then incubated at 37 °C for 72 h in a humidified environment containing 5% CO_2. The tetrazolium dye-based CellTiter reagent (Promega, Madison, WI) was used to assess parasite growth.[96] Several hours after adding 12 μL of the CellTiter reagent to each well of the plate, absorbance readings were taken at 490 nm using a SpectraMax Plus 384 microplate reader (Molecular Devices, Sunnyvale, CA). SoftMax Pro software (Amersham Biosciences, Piscataway, NJ) was used to calculate IC_{50} values by employing the dose–response equation $y = [(a - d)/(1 + (x/c)^b)] + d$, where x = compound concentration, y = absorbance at 490 nm, a = upper asymptote, b = slope, c = IC_{50} value, and d = lower asymptote.

STIB900 Acute Mouse Model of Trypanosomiasis—Experiments were performed as previously reported[97] with minor modifications. Briefly, female NMRI mice were infected intraperitoneally (ip) with 2 × 10^4 STIB900 bloodstream forms. Experimental groups of four mice were treated ip with tested dications on 4 consecutive days from day 3 to day 6 postinfection. A control group was infected but remained untreated. After drug treatment, parasitamia of all animals was checked by tail blood examination on day 7, day 10, then twice a week until day 30 followed by once a week until 60 days postinfection. After detection of parasitamia, the day of parasitamia relapse was recorded to calculate the MRD and mice were euthanized. Surviving and aparasitemic mice at day 60 were considered cured and then euthanized.

Chemistry

General Experimental Information: All chemicals and solvents were purchased from Aldrich Chemical Co., Fisher Scientific, or Acros Organics and were used without further purification. The purity of all novel compounds was confirmed to exceed 95% by elemental analysis and HPLC. Uncorrected melting points were measured on a Thomas–Hoover capillary melting point apparatus. ^{1}H NMR spectra were recorded on a Varian Gemini 2000 spectrometer operating at 300 MHz. Chemical shifts are reported in ppm relative to tetramethylsilane. Anhydrous ethanol was distilled over Mg/I_2 immediately prior to use. Reaction mixtures were monitored by TLC using Whatman silica gel 250 μm UV_{254} plates or by reversed phase HPLC. Organic layers of extraction mixtures were washed with saturated NaCl solution and dried over Na_2SO_4 or $MgSO_4$ before being evaporated under reduced pressure. Flash column chromatography was performed using Davisil grade 633, type 60A silica gel (200–425 mesh). Analytical HPLC chromatograms were recorded on an Agilent 1200 chromatograph using an Agilent Zorbax SB C8 column (4.6 mm × 75 mm, 3.5 μm) and UV photodiode array detection at 230, 254, 265, 290, and 320 nm. The column

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temperature was maintained at 40 °C. Mobile phases consisted of mixtures of methanol (0–95%) and water, both solvents containing formic acid (80 mM), ammonium formate (20 mM), and triethylamine (15 mM). Flow rates were maintained at 1.5 mL/min. In method A, the concentration of methanol was increased linearly from 0 to 28.5% over 6 min, from 28.5 to 71.25% over 4 min, from 71.25 to 95% over 0.5 min, and maintained at 95% for 2 min before re-equilibration. In method B, the concentration of methanol was increased linearly from 28.5 to 95% over 10 min and then maintained at 95% for 2 min before re-equilibration.

Preparative Reversed Phase HPLC—Preparative reversed phase HPLC was performed on a Varian ProStar Chromatography Workstation configured with two PS-215 pumps fitted with 50 mL pump heads, a Dynamax Microsorb C18 (60 Å) column (41.4 mm × 250 mm, 8 μm), PS-320 variable wavelength UV–vis detector, and a PS-701 fraction collector. Mobile phases consisted of mixtures of acetonitrile (0–75%) in water containing formic acid (40 mM) and ammonium formate (10 mM). Flow rates were maintained at 40 mL/min. Detector wavelengths and mobile phase gradients were optimized for the individual compounds. Select fractions were analyzed for purity as described above for analytical HPLC. Residues of evaporated pooled purified fractions were reconstituted in water and lyophilized on a VirTis BenchTop 6K lyophilizer. The lyophilized compounds were dissolved in ethanol and converted into HCl salts with aqueous HCl.

Flash Chromatography of Amidines on C_{18} Reversed Phase Silica Gel—The chromatographic column was half-filled with acetonitrile and packed with a slurry of C_{18} Silica Gel (70 g) in acetonitrile (70–100 mL). The excess acetonitrile was drained out, and the top of the column was covered with a 2 cm pad of sand. The column was equilibrated with 150 mL of initial mobile phase consisting of water containing formic acid (40 mM) and ammonium formate (10 mM). A concentrated reaction mixture was dissolved in the initial mobile phase. In case of low solubility, heating of the mixture and/or addition of a small amount of methanol as a cosolvent were performed. After the reaction mixture was applied to the column, the elution began with initial mobile phase (150 mL) to remove the excess amine and then with a mobile phase consisting of a mixture of acetonitrile (0–75%) in water containing formic acid (40 mM) and ammonium formate (10 mM). Acetonitrile concentrations varied for each individual compound and contained 50–70% of calculated amount of acetonitrile at the point of the retention time of the compound in analytical method A. After the purification was completed, the column was washed with acetonitrile (3 × 100 mL), ethanol (100 mL), deionized water (2 × 100 mL), and kept in acetonitrile or acetonitrile–water mixture. Select fractions were analyzed for purity as described above for analytical HPLC. Residues of evaporated pooled purified fractions were reconstituted in water and lyophilized on a VirTis BenchTop 6K lyophilizer. The lyophilized compounds were dissolved in ethanol and converted into HCl salts with aqueous HCl.

Low resolution ESI mass spectra were recorded on an Agilent Technologies 1100 series LC/MSD trap spectrometer. Elemental analyses were performed by Atlantic Microlab, Norcross, GA, and were within ±0.4% of calculated values.

General Procedure for Synthesis of Dications (1–60). 1,4-Bis(3-amidinophenyl)-1*H*-1,2,3-triazole Dihydrochloride (1)—A mixture of dry 1,4-dioxane (60 mL) and dry EtOH (15 mL) in a three-neck 250 mL flask equipped with a gas inlet tube, a thermometer, and a drying tube was saturated with gaseous HCl at 0 °C. 1,4-Bis(3-cyanophenyl)-1*H*-1,2,3-triazole (**77**) (1.51 g, 5.57 mmol) was added in one portion, the flask was sealed, and the mixture was stirred at ambient temperature until the starting material was no longer detectable by HPLC. The reaction mixture was diluted with dry ether (100 ml). A formed precipitate was filtered off under argon, washed with diethyl ether, and

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dried under high vacuum over KOH to give diimidate ester (2.27 g, 94%), which was split in three portions and reacted immediately with the appropriate amines.

To a suspension of the diimidate ester (0.75 g, 1.72 mmol) in dry EtOH (20 ml) was added saturated ethanolic ammonia (10 mL). The reaction mixture was sealed and stirred at ambient temperature. The progress of the reaction was monitored by HPLC. After four days, the mixture was diluted with dry diethyl ether and cooled in a freezer. A resulting precipitate was filtered off, washed with ether and dried under vacuum to give 0.49 g of crude material, which was purified by reverse phase column chromatography and recrystallized from 1.5 N HCl to give **1**, as a white solid (0.21 g, 32%); mp > 350 °C (dec). ^{1}H NMR (DMSO-d_6) δ 9.82 (s, 1H), 9.74 (br s, 2H), 9.60 (br s, 2H), 9.47 (br s, 2H), 9.36 (br s, 2H), 8.58 (s, 1H), 8.50 (s, 1H), 8.34 (d, J = 7.7 Hz, 1H), 8.26 (d, J = 7.7 Hz, 1H), 8.00 (d, J = 7.7 Hz, 1H), 7.91 (dd, J = 7.7 and 7.7 Hz, 1H), 7.87 (d, J = 7.7 Hz, 1H), 7.78 (dd, J = 7.7 and 7.7 Hz, 1H). HPLC (method A) t_R 5.12 min (100 area %). *M/Z* 306.8 (MH$^+$ of free base). Anal. ($C_{16}H_{15}N_7$·2HCl·1.3H_2O) C, H, N, Cl.

1,4-Bis(3-*N*-isopropylamidinophenyl)-1*H*-1,2,3-triazole Dihydrochloride (2)— White solid (0.36 g, 45%); mp 265–267 °C (dec). ^{1}H NMR (DMSO-d_6) δ 9.90 (s, 1H), 9.86 (br s, 6H), 8.48 (s, 1H), 8.37 (s, 1H), 8.32 (d, J = 7.7 Hz, 1H), 8.25 (m, 1H), 7.92 (d, J = 7.7 Hz, 1H), 7.88 (dd, J = 7.7 and 7.7 Hz, 1H), 7.77 (d, J = 7.7 Hz, 1H), 7.75 (dd, J = 7.7 and 7.7 Hz, 1H), 4.18 (m, 2H), 1.33 (d, J = 6.7 Hz, 6H), 1.31 (d, J = 6.7 Hz, 6H). HPLC (method A) t_R 7.41 min (100 area %). *M/Z* 390.8 (MH$^+$ of free base). Anal. ($C_{22}H_{27}N_7$·2HCl·1.5H_2O) C, H, N, Cl.

1,4-Bis[3-(4,5-dihydro-1*H*-imidazol-2-yl)phenyl]-1*H*-1,2,3-triazole Dihydrochloride (3)—White solid (0.35 g, 45%); mp 353–355 °C (dec). ^{1}H NMR (DMSO-d_6) δ 11.1 (br s, 4H), 9.82 (s, 1H), 8.94 (s, 1H), 8.81 (s, 1H), 8.34 (d, J = 8.2 Hz, 1H), 8.25 (d, J = 7.7 Hz, 1H), 8.24 (d, J = 7.7 Hz, 1H), 8.09 (d, J = 8.2 Hz, 1H), 7.94 (dd, J = 8.2 and 8.2 Hz, 1H), 7.81 (dd, J = 7.7 and 7.7 Hz, 1H), 4.06 (s, 8H). HPLC (method A) t_R 6.79 min (100 area %). *M/Z* 358.8 (MH$^+$ of free base). Anal. ($C_{20}H_{19}N_7$·2HCl·1.5H_2O) C, H, N, Cl.

1-(5-Amidino-2-methoxyphenyl)-4-(3-amidinophenyl)-1*H*-1,2,3-triazole Dihydrochloride (4)—White solid (0.63 g, 60%); mp > 243 °C (dec). ^{1}H NMR (DMSO-d_6) δ 9.60 (br s, 2H), 9.51 (br s, 2H), 9.30 (br s, 2H), 9.24 (s, 1H), 9.23 (br s, 2H), 8.50 (s, 1H), 8.32 (d, J = 1.8 Hz, 1H), 8.29 (d, J = 8.8 Hz, 1H), 8.14 (dd, J = 8.8 and 1.8 Hz, 1H), 7.84 (d, J = 7.7 Hz, 1H), 7.76 (dd, J = 7.7 and 7.7 Hz, 1H), 7.62 (d, J = 7.7 Hz, 1H), 4.03 (s, 3H). HPLC (method A) t_R 5.97 min (100 area %). *M/Z* 336.2 (MH$^+$ of free base). Anal. ($C_{17}H_{17}N_7O$·2HCl·3H_2O) C, H, N, Cl.

1-(5-*N*-Isopropylamidino-2-methoxyphenyl)-4-(3-*N*-isopropylamidinophenyl)-1*H*-1,2,3-triazole Dihydrochloride (5)—White solid (0.61 g, 48%); mp > 248 °C (dec). ^{1}H NMR (DMSO-d_6) δ 9.83 (d, J = 6.0 Hz, 1H), 9.75 (d, J = 6.8 Hz, 1H), 9.67 (br s, 1H), 9.62 (br s, 1H), 9.31 (br s, 1H), 9.29 (s, 1H), 9.22 (br s, 1H), 8.39 (s, 1H), 8.27 (d, J = 8.8 Hz, 1H), 8.18 (d, J = 2.2 Hz, 1H), 8.02 (dd, J = 8.8 and 2.2 Hz, 1H), 7.76 (m, 2H), 7.59 (d, J = 8.8 Hz, 1H), 4.13 (m, 2H), 4.01 (s, 3H), 1.30 (s, 12H). HPLC (method A) t_R 7.84 min (100 area %). *M/Z* 420.6 (MH$^+$ of free base). Anal. ($C_{23}H_{29}N_7O$·2.4HCl·2.6H_2O) C, H, N, Cl.

1-[5-(4,5-Dihydro-1*H*-imidazol-2-yl)-2-methoxyphenyl]-4-[3-(4,5-dihydro-1*H*-imidazol-2-yl)phenyl]-1*H*-1,2,3-triazole Dihydrochloride (6)—White solid (0.78 g, 65%); mp > 250 °C (dec). ^{1}H NMR (DMSO-d_6) δ 11.15 (br s, 2H), 10.93 (br s, 2H), 9.34 (s, 1H), 8.96 (s, 1H), 8.57 (s, 1H), 8.40 (d, J = 8.2 Hz, 1H), 8.35 (d, J = 7.7 Hz, 1H), 8.10 (d, J

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= 8.0 Hz, 1H), 7.79 (dd, *J* = 8.0 and 7.7 Hz, 1H), 7.68 (d, *J* = 8.8 Hz, 1H), 4.06 (s, 4H), 4.04 (s, 4H), 4.01 (s, 3H). HPLC (method A) t_R 7.43 min (100 area %). *M/Z* 388.3 (MH^+ of free base). Anal. ($C_{21}H_{21}N_7O{\cdot}2.5HCl{\cdot}2.5H_2O$) C, H, N, Cl.

4-(5-Amidino-2-methoxyphenyl)-1-(3-amidinophenyl)-1*H*-1,2,3-triazole Dihydrochloride (7)—White solid (0.58 g, 70%); mp > 246 °C (dec). ^{1}H NMR (DMSO-d_6) δ 9.82 (br s, 2H), 9.48 (br s, 4H), 9.46 (s, 1H), 9.17 (br s, 2H), 8.72 (d, *J* = 2.3 Hz, 1H), 8.62 (s, 1H), 8.40 (d, *J* = 8.0 Hz, 1H), 7.99 (d, *J* = 8.0 Hz, 1H), 7.93 (dd, *J* = 8.8 and 2.3 Hz, 1H), 7.88 (dd, *J* = 8.0 and 8.0 Hz, 1H), 7.45 (d, *J* = 8.8 Hz, 1H), 4.14 (s, 3H). HPLC (method A) t_R 6.44 min (100 area %). *M/Z* 336.5 (MH^+ of free base). Anal. ($C_{17}H_{17}N_7O{\cdot}2HCl{\cdot}2.3H_2O$) C, H, N, Cl.

4-(5-*N*-Isopropylamidino-2-methoxyphenyl)-1-(3-*N*-isopropylamidinophenyl)-1*H*-1,2,3-triazole Dihydrochloride (8)—White solid (0.66 g, 66%); mp > 275 °C (dec). ^{1}H NMR (DMSO-d_6) δ 10.14 (br s, 1H), 9.93 (br s, 1H), 9.63 (d, *J* = 6 Hz, 1H), 9.54 (s, 1H), 9.50 (br s, 1H), 9.42 (br s, 1H), 9.13 (br s, 1H), 8.59 (d, *J* = 2.3 Hz, 1H), 8.52 (s, 1H), 8.35 (d, *J* = 7.8 Hz, 1H), 7.91 (d, *J* = 7.8 Hz, 1H), 7.86 (dd, *J* = 7.8 and 7.8 Hz, 1H), 7.82 (dd, *J* = 8.8 and 2.3 Hz, 1H), 7.42 (d, *J* = 8.8 Hz, 1H), 4.14 (m, 2H), 4.13 (s, 3H), 1.33 (d, *J* = 6.5 Hz, 6H), 1.31 (d, *J* = 6.5 Hz, 6H). HPLC (method A) t_R 8.24 min (100 area %). *M/Z* 420.6 (MH^+ of free base). Anal. ($C_{23}H_{29}N_7O{\cdot}2HCl{\cdot}1.1H_2O$) C, H, N, Cl.

4-[5-(4,5-Dihydro-1*H*-imidazol-2-yl)-2-methoxyphenyl]-1-[3-(4,5-dihydro-1*H*-imidazol-2-yl)phenyl]-1*H*-1,2,3-triazole Dihydrochloride (9)—White solid (0.64 g, 69%); mp > 235 °C (dec). ^{1}H NMR (DMSO-d_6) δ 11.38 (br s, 2H), 10.74 (br s, 2H), 9.53 (s, 1H), 9.08 (s, 1H), 8.87 (d, *J* = 2.3 Hz, 1H), 8.48 (d, *J* = 8.0 Hz, 1H), 8.22 (d, *J* = 8.0 Hz, 1H), 8.16 (dd, *J* = 8.8 and 2.3 Hz, 1H), 7.92 (dd, *J* = 8.0 and 8.0 Hz, 1H), 7.48 (d, *J* = 8.8 Hz, 1H), 4.18 (s, 3H), 4.06 (s, 4H), 4.01 (s, 4H). HPLC (method A) t_R 7.83 min (100 area %). *M/Z* 388.8 (MH^+ of free base). Anal. ($C_{21}H_{21}N_7O{\cdot}2HCl{\cdot}2.9H_2O$) C, H, N, Cl.

1-(5-Amidino-2-hydroxyphenyl)-4-(3-amidinophenyl)-1*H*-1,2,3-triazole Dihydrochloride (10)—Brown solid (0.30 g, 41%); mp 260 °C (dec). ^{1}H NMR (DMSO-d_6) δ 12.29 (s, 1H), 9.57 (s, 2H), 9.39 (s, 2H), 9.29 (s, 2H), 9.22 (s, 1H), 9.12 (s, 2H), 8.48 (s, 1H), 8.32 (d, *J* = 7.7 Hz, 1H), 8.28 (s, 1H), 7.94 (d, *J* = 8.2 Hz, 1H), 7.84 (d, *J* = 7.7 Hz, 1H), 7.75 (dd, *J* = 7.7 and 7.7 Hz, 1H), 7.49 (d, *J* = 8.2 Hz, 1H). HPLC (method A) t_R 5.09 min (100 area %). *M/Z* 322.2 (MH^+ of free base). Anal. ($C_{16}H_{15}N_7O{\cdot}2HCl{\cdot}2H_2O$) C, H, N, Cl.

1-(5-*N*-Isopropylamidino-2-hydroxyphenyl)-4-(3-*N*-isopropylamidinophenyl)-1*H*-1,2,3-triazole Dihydrochloride (11)—Brown solid (0.26 g, 29%); mp > 245 °C (dec). ^{1}H NMR (DMSO-d_6) δ 12.24 (s, 1H), 9.79 (d, *J* = 7.7 Hz, 1H), 9.62 (br s, 1H), 9.60 (d, *J* = 7.0 Hz, 1H), 9.48 (br s, 1H), 9.28 (br s, 1H), 9.25 (s, 1H), 9.10 (br s, 1H), 8.36 (s, 1H), 8.30 (d, *J* = 1.8 Hz, 1H), 8.12 (s, 1H), 7.83 (d, *J* = 7.7 Hz, 1H), 7.74 (br s, 2H), 7.52 (d, *J* = 8.8 Hz, 1H), 4.11 (m, 2H), 1.31 (d, *J* = 6.4 Hz, 6H), 1.28 (d, *J* = 6.4 Hz, 6H). HPLC (method A) t_R 7.57 min (100 area %). *M/Z* 406.2 (MH^+ of free base). Anal. ($C_{22}H_{27}N_7O{\cdot}2.3HCl{\cdot}2.8H_2O$) C, H, N, Cl.

1-[5-(4,5-Dihydro-1*H*-imidazol-2-yl)-2-hydroxyphenyl]-4-[3-(4,5-dihydro-1*H*-imidazol-2-yl)phenyl]-1*H*-1,2,3-triazole Dihydrochloride (12)—Brown solid (0.41 g, 49%); mp 247–250 °C (dec). ^{1}H NMR (DMSO-d_6) δ 12.26 (s, 1H), 11.07 (s, 2H), 10.76 (s, 2H), 9.26 (s, 1H), 8.85 (s, 1H), 8.49 (s, 1H), 8.36 (d, *J* = 8.2 Hz, 1H), 8.18 (d, *J* = 8.8 Hz, 1H), 8.09 (d, *J* = 7.7 Hz, 1H), 7.78 (dd, *J* = 7.7 and 7.7 Hz, 1H), 7.55 (d, *J* = 8.8 Hz, 1H),

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4.04 (s, 4H), 3.98 (s, 4H). HPLC (method A) t_R 7.00 min (100 area %). *M/Z* 374.2 (MH^+ of free base). Anal. ($C_{20}H_{19}N_7O{\cdot}2.5HCl{\cdot}3H_2O$) C, H, N, Cl.

4-(5-Amidino-2-hydroxyphenyl)-1-(3-amidinophenyl)-1*H*-1,2,3-triazole Dihydrochloride (13)—Purple solid (0.23 g, 41%); mp > 278 °C (dec). ^{1}H NMR (DMSO-d_6) δ 11.87 (s, 1H), 9.73 (br s, 2H), 9.45 (br s, 2H), 9.35 (br s, 2H), 9.27 (s, 1H), 9.05 (br s, 2H), 8.68 (d, *J* = 2.4 Hz, 1H), 8.51 (s, 1H), 8.41 (d, *J* = 8.0 Hz, 1H), 7.98 (d, *J* = 8.0 Hz, 1H), 7.88 (d, *J* = 8.0 Hz, 1H), 7.78 (dd, *J* = 8.7 and 2.4 Hz, 1H), 7.39 (d, *J* = 8.7 Hz, 1H). HPLC (method A) t_R 5.64 min (100 area %). *M/Z* 322.2 (MH^+ of free base). Anal. ($C_{16}H_{15}N_7O{\cdot}2HCl{\cdot}1.5H_2O$) C, H, N, Cl.

4-(5-*N*-Isopropylamidino-2-hydroxyphenyl)-1-(3-*N*-isopropylamidinophenyl)-1*H*-1,2,3-triazole Dihydrochloride (14)—Purple solid (0.26 g, 37%); mp > 245 °C (dec). ^{1}H NMR (DMSO-d_6) δ 11.80 (s, 1H), 9.93 (d, *J* = 8.8 Hz, 1H), 9.76 (br s, 1H), 9.51 (d, *J* = 8.9 Hz, 1H), 9.38 (br s, 2H), 9.27 (s, 1H), 9.00 (br s, 1H), 8.52 (d, *J* = 2.2 Hz, 1H), 8.40 (s, 1H), 8.35 (d, *J* = 8.7 Hz, 1H), 7.87 (d, *J* = 8.2 Hz, 1H), 7.85 (m, 1H), 7.63 (dd, *J* = 8.8 and 2.2Hz, 1H), 7.39 (d, *J* = 8.8Hz, 1H), 4.12 (m, 2H), 1.33 (d, *J* = 6.4 Hz, 6H), 1.31 (d, *J* = 6.4 Hz, 6H). HPLC (method A) t_R 7.97 min (100 area %). *M/Z* 406.2 (MH^+ of free base). Anal. ($C_{22}H_{27}N_7O{\cdot}2.4HCl{\cdot}2H_2O$) C, H, N, Cl.

4-[5-(4,5-Dihydro-1*H*-imidazol-2-yl)-2-hydroxyphenyl]-1-[3-(4,5-dihydro-1*H*-imidazol-2-yl)phenyl]-1*H*-1,2,3-triazole Dihydrochloride (15)—White solid (0.19 g, 29%); mp > 307 °C (dec). ^{1}H NMR (DMSO-d_6) δ 11.33 (s, 1H), 10.64 (br s, 4H), 9.29 (s, 1H), 8.85 (s, 1H), 8.80 (d, *J* = 2.4 Hz, 1H), 8.43 (d, *J* = 8.0 Hz, 1H), 8.22 (d, *J* = 8.0 Hz, 1H), 8.05 (dd, *J* = 8.7 and 2.4 Hz, 1H), 7.91 (dd, *J* = 8.0 and 8.0 Hz, 1H), 7.40 (d, *J* = 8.7 Hz, 1H), 4.06 (s, 4H), 3.99 (s, 4H). HPLC (method A) t_R 7.51 min (100 area %). *M/Z* 374.2 (MH^+ of free base). Anal. ($C_{20}H_{19}N_7O{\cdot}2HCl{\cdot}1.7H_2O{\cdot}0.3EtOH$) C, H, N, Cl.

1-(4-Amidinophenyl)-4-(3-amidinophenyl)-1*H*-1,2,3-triazole Dihydrochloride (16)—White solid (0.33 g, 57%); mp 274–277 °C (dec). ^{1}H NMR (DMSO-d_6) δ 9.80 (s, 1H), 9.62 (br s, 4H), 9.39 (br s, 4H), 8.55 (s, 1H), 8.29 (d, *J* = 8.2 Hz, 1H), 8.25 (d, *J* = 8.8 Hz, 2H), 8.15 (d, *J* = 8.8 Hz, 2H), 7.88 (d, *J* = 8.2 Hz, 1H), 7.81 (dd, *J* = 8.2 and 8.2 Hz, 1H). HPLC (method A) t_R 5.25 min (100 area %). Anal. ($C_{16}H_{15}N_7{\cdot}2HCl{\cdot}1.2H_2O$) C, H, N, Cl.

1-(4-*N*-Isopropylamidinophenyl)-4-(3-*N*-isopropylamidinophenyl)-1*H*-1,2,3-triazole Dihydrochloride (17)—White solid (0.33 g, 45%); mp 335–336 °C (dec). ^{1}H NMR (DMSO-d_6) δ 9.84 (s, 1H), 9.70 (m, 6H), 8.40 (s, 1H), 8.28 (m, 1H), 8.24 (d, *J* = 8.8 Hz, 2H), 8.04 (d, *J* = 8.8 Hz, 2H), 7.76 (m, 2H), 4.14 (m, 2H), 1.32 (d, *J* = 6.6 Hz, 6H), 1.30 (d, *J* = 6.6 Hz, 6H). HPLC (method A) t_R 7.65 min (100 area %). Anal. ($C_{22}H_{27}N_7{\cdot}2HCl{\cdot}0.6H_2O$) C, H, N, Cl.

1-[4-(4,5-Dihydro-1*H*-imidazol-2-yl)phenyl]-4-(3-(4,5-dihydro-1*H*-imidazol-2-yl)phenyl)-1*H*-1,2,3-triazole Dihydrochloride (18)—Off-white solid (0.34 g, 51%); mp > 272 °C (dec). ^{1}H NMR (DMSO-d_6) δ 11.1 (br s, 4H), 9.82 (s, 1H), 8.90 (s, 1H), 8.38 (d, *J* = 8.2 Hz, 2H), 8.29 (d, *J* = 8.2 Hz, 1H), 8.26 (d, *J* = 8.2 Hz, 2H), 8.10 (d, *J* = 7.7 Hz, 1H), 7.82 (dd, *J* = 8.2 and 7.7 Hz, 1H), 4.05 (s, 8H). HPLC (method A) t_R 6.94 min (100 area %). Anal. ($C_{20}H_{19}N_7{\cdot}2.2HCl{\cdot}1.6H_2O$) C, H, N, Cl.

1-(4-Amidino-2-methoxyphenyl)-4-(3-amidinophenyl)-1*H*-1,2,3-triazole Dihydrochloride (19)—White solid (0.55 g, 52%); mp 127–130 °C. ^{1}H NMR (DMSO-d_6) δ 9.73 (br s, 2H), 9.62 (br s, 2H), 9.44 (br s, 2H), 9.34 (br s, 2H), 9.30 (s, 1H), 8.53 (s, 1H),

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8.32 (d, *J* = 7.7 Hz, 1H), 8.02 (d, *J* = 8.3 Hz, 1H), 7.88 (d, *J* = 1.9 Hz, 1H), 7.86 (d, *J* = 7.7 Hz, 1H), 7.76 (dd, *J* = 7.7 and 7.7 Hz, 1H), 7.66 (dd, *J* = 8.3 and 1.9 Hz, 1H), 4.06 (s, 3H). HPLC (method A) t_R 5.89 min (100 area %). *M/Z* 336.2 (MH^+ of free base). Anal. ($C_{17}H_{17}N_7O{\cdot}2.2HCl{\cdot}3H_2O$) C, H, N, Cl.

1-(4-*N*-Isopropylamidino-2-methoxyphenyl)-4-(3-*N*-isopropylamidinophenyl)-1*H*-1,2,3-triazole Dihydrochloride (20)—Off-white solid (0.60 g, 47%); mp 80–83 °C (dec). ^{1}H NMR (DMSO-d_6) δ 9.95 (d, *J* = 7.2 Hz, 1H), 9.81 (d, *J* = 7.0 Hz, 1H), 9.80 (br s, 1H), 9.64 (br s, 1H), 9.40 (br s, 1H), 9.30 (s, 1H), 9.29 (br s, 1H), 8.40 (s, 1H), 8.29 (m, 1H), 7.98 (d, *J* = 8.3 Hz, 1H), 7.78 (br s, 1H), 7.75 (m, 1H), 7.73 (dd, *J* = 7.7 and 7.7 Hz, 1H), 7.55 (dd, *J* = 8.3 and 1.6 Hz, 1H), 4.14 (m, 2H), 4.06 (s, 3H), 1.33 (d, *J* = 6.2 Hz, 6H), 1.31 (d, *J* = 6.2 Hz, 6H). HPLC (method A) t_R 7.96 min (100 area %). *M/Z* 420.2 (MH^+ of free base). Anal. ($C_{23}H_{29}N_7O{\cdot}2.5HCl{\cdot}3H_2O$) C, H, N, Cl.

1-[4-(4,5-Dihydro-1*H*-imidazol-2-yl)-2-methoxyphenyl]-4-[3-(4,5-dihydro-1*H*-imidazol-2-yl)phenyl]-1*H*-1,2,3-triazole Dihydrochloride (21)—Off-white solid (0.82 g, 69%); mp > 135 °C (dec). ^{1}H NMR (DMSO-d_6) δ 11.24 (s, 2H), 11.08 (s, 2H), 9.38 (s, 1H), 8.91 (s, 1H), 8.35 (d, *J* = 7.7 Hz, 1H), 8.23 (d, *J* = 1.6 Hz, 1H), 8.10 (d, *J* = 8.3 Hz, 1H), 8.07 (d, *J* = 7.7 Hz, 1H), 7.88 (dd, *J* = 8.3 and 1.6 Hz, 1H), 7.79 (dd, *J* = 7.7 and 7.7 Hz, 1H), 4.08 (s, 3H), 4.06 (s, 4H), 4.05 (s, 4H). HPLC (method A) t_R 7.48 min (100 area %). *M/Z* 388.3 (MH^+ of free base). Anal. ($C_{21}H_{21}N_7O{\cdot}2.2HCl{\cdot}3H_2O$) C, H, N, Cl.

4-(5-Amidino-2-methoxyphenyl)-1-(4-amidinophenyl)-1*H*-1,2,3-triazole Dihydrochloride (22)—White solid (0.53 g, 65%); mp 270–272 °C (dec). ^{1}H NMR (DMSO-d_6) δ 9.62 (br s, 2H), 9.45 (br s, 4H), 9.30 (s, 1H), 9.20 (br s, 2H), 8.72 (d, *J* = 2.2 Hz, 1H), 8.35 (d, *J* = 8.8 Hz, 2H), 8.14 (d, *J* = 8.8 Hz, 2H), 7.96 (dd, *J* = 8.8 and 2.2 Hz, 1H), 7.45 (d, *J* = 8.8 Hz, 1H), 4.12 (s, 3H). HPLC (method A) t_R 6.38 min (100 area %). Anal. ($C_{17}H_{17}N_7O{\cdot}2HCl{\cdot}2.1H_2O{\cdot}0.2EtOH$) C, H, N, Cl.

4-(5-*N*-Isopropylamidino-2-methoxyphenyl)-1-(4-*N*-isopropylamidinophenyl)-1*H*-1,2,3-triazole Dihydrochloride (23)—White solid (0.18 g, 19%); mp 283–284 °C (dec). ^{1}H NMR (DMSO-d_6) δ 9.62 (br s, 4H), 9.30 (s, 1H), 9.15 (br s, 2H), 8.58 (d, *J* = 2.2 Hz, 1H), 8.33 (d, *J* = 8.8 Hz, 2H), 8.03 (d, *J* = 8.8 Hz, 2H), 7.83 (dd, *J* = 8.8 and 2.2 Hz, 1H), 7.42 (d, *J* = 8.8 Hz, 1H), 4.17 (m, 2H), 4.11 (s, 3H), 1.32 (d, *J* = 6.6 Hz, 6H), 1.30 (d, *J* = 6.6 Hz, 6H). HPLC (method A) t_R 8.31 min (100 area %). Anal. ($C_{23}H_{29}N_7O{\cdot}2HCl{\cdot}0.9H_2O$) C, H, N, Cl.

4-[5-(4,5-Dihydro-1*H*-imidazol-2-yl)-2-methoxyphenyl]-1-[4-(4,5-dihydro-1*H*-imidazol-2-yl)phenyl]-1*H*-1,2,3-triazole Dihydrochloride (24)—White solid (0.50 g, 55%); mp 315–317 °C (dec). ^{1}H NMR (DMSO-d_6) δ 10.9 (br s, 2H), 10.6 (br s, 2H), 9.31 (s, 1H), 8.87 (br s, 1H), 8.41 (d, *J* = 8.8 Hz, 2H), 8.29 (d, *J* = 8.8 Hz, 2H), 8.10 (dd, *J* = 8.2 and 8.2 Hz, 1H), 7.51 (d, *J* = 8.2 Hz, 1H), 4.13 (s, 3H), 4.06 (s, 4H), 4.01 (s, 4H). HPLC (method A) t_R 7.88 min (100 area %). Anal. ($C_{21}H_{21}N_7O{\cdot}2HCl{\cdot}1H_2O$) C, H, N, Cl.

1-(4-Amidino-2-hydroxyphenyl)-4-(3-amidinophenyl)-1*H*-1,2,3-triazole Dihydrochloride (25)—Light-brown solid (0.91 g, 70%); mp > 230 °C (dec). ^{1}H NMR (DMSO-d_6) δ 11.83 (br s, 1H), 9.57 (br s, 4H), 9.33 (br s, 4H), 9.26 (s, 1H), 8.50 (s, 1H), 8.34 (d, *J* = 7.8 Hz, 1H), 7.95 (d, *J* = 8.2 Hz, 1H), 7.85 (d, *J* = 7.8 Hz, 1H), 7.75 (dd, *J* = 7.8 and 7.8 Hz, 1H), 7.66 (s, 1H), 7.42 (d, *J* = 8.2 Hz, 1H). HPLC (method A) t_R 5.39 min (100 area %). *M/Z* 322.2 (MH^+ of free base). Anal. ($C_{16}H_{15}N_7O{\cdot}2HCl{\cdot}2H_2O$) C, H, N, Cl.

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1-(4-*N*-Isopropylamidino-2-hydroxyphenyl)-4-(3-*N*-isopropylamidinophenyl)-1*H*-1,2,3-triazole Dihydrochloride (26)—Grey solid (0.55 g, 35%); mp > 235 °C (dec). ^{1}H NMR (DMSO-d_6) δ 11.84 (s, 1H), 9.78 (br s, 2H), 9.60 (br s, 2H), 9.25 (br s, 3H), 8.36 (s, 1H), 8.32 (m, 1H), 7.92 (d, *J* = 7.7 Hz, 1H), 7.73 (m, 2H), 7.61 (s, 1H), 7.34 (d, *J* = 8.2 Hz, 1H), 4.11 (m, 2H), 1.31 (d, *J* = 6.2 Hz, 6H), 1.29 (d, *J* = 6.2 Hz, 6H). HPLC (method A) t_R 7.71 min (100 area %). *M/Z* 406.3 (MH$^+$ of free base). Anal. ($C_{22}H_{27}N_7O{\cdot}2HCl{\cdot}2H_2O$) C, H, N, Cl.

1-[4-(4,5-Dihydro-1*H*-imidazol-2-yl)-2-hydroxyphenyl]-4-[3-(4,5-dihydro-1*H*-imidazol-2-yl)phenyl]-1*H*-1,2,3-triazole Dihydrochloride (27)—Yellow solid (0.75 g, 51%); mp > 250 °C (dec). ^{1}H NMR (DMSO-d_6) δ 10.96 (br s, 4H), 9.31 (s, 1H), 8.85 (s, 1H), 8.37 (d, *J* = 7.7 Hz, 1H), 8.07 (d, *J* = 7.7 Hz, 1H), 8.02 (d, *J* = 8.3 Hz, 1H), 7.80 (d, *J* = 7.7 Hz, 1H), 7.74 (m, 1H), 7.62 (d, *J* = 8.3 Hz, 1H), 4.04 (s, 8H). HPLC (method A) t_R 7.17 min (100 area %). *M/Z* 374.2 (MH$^+$ of free base). Anal. ($C_{20}H_{19}N_7O{\cdot}2HCl{\cdot}2H_2O$) C, H, N, Cl.

4-(5-Amidino-2-hydroxyphenyl)-1-(4-amidinophenyl)-1*H*-1,2,3-triazole Dihydrochloride (28)—Purple solid (0.54 g, 57%); mp > 251 °C (dec). ^{1}H NMR (DMSO-d_6) δ 11.80 (s, 1H), 9.61 (br s, 2H), 9.36 (br s, 2H), 9.33 (br s, 2H), 9.22 (s, 1H), 9.03 (br s, 2H), 8.65 (d, *J* = 2.5 Hz, 1H), 8.31 (d, *J* = 8.8 Hz, 2H), 8.11 (d, *J* = 8.8 Hz, 2H), 7.76 (dd, *J* = 8.6 and 2.5 Hz, 1H), 7.36 (d, *J* = 8.6 Hz, 1H). HPLC (method A) t_R 5.74 min (100 area %). *M/Z* 322.7 (MH$^+$ of free base). Anal. ($C_{16}H_{15}N_7O{\cdot}2HCl{\cdot}0.9H_2O$) C, H, N, Cl.

4-(5-*N*-Isopropylamidino-2-hydroxyphenyl)-1-(4-*N*-isopropylamidinophenyl)-1*H*-1,2,3-triazole Dihydrochloride (29)—White solid (0.66 g, 57%); mp > 255 °C (dec). ^{1}H NMR (DMSO-d_6) δ 11.77 (s, 1H), 9.83 (d, *J* = 8.8 Hz, 1H), 9.67 (br s, 1H), 9.53 (d, *J* = 7.6 Hz, 1H), 9.39 (br s, 1H), 9.33 (br s, 1H), 9.22 (s, 1H), 9.01 (br s, 1H), 8.51 (d, *J* = 2.3 Hz, 1H), 8.30 (d, *J* = 8.6 Hz, 2H), 8.00 (d, *J* = 8.6 Hz, 2H), 7.65 (dd, *J* = 8.7 and 2.3 Hz, 1H), 7.40 (d, *J* = 8.8 Hz, 1H), 4.11 (m, 2H), 1.31 (d, *J* = 6.2 Hz, 6H), 1.29 (d, *J* = 6.2 Hz, 6H). HPLC (method A) t_R 8.10 min (100 area %). *M/Z* 406.7 (MH$^+$ of free base). Anal. ($C_{22}H_{27}N_7O{\cdot}2HCl{\cdot}1.4H_2O$) C, H, N, Cl.

4-[5-(4,5-Dihydro-1*H*-imidazol-2-yl)-2-hydroxyphenyl]-1-[4-(4,5-dihydro-1*H*-imidazol-2-yl)phenyl]-1*H*-1,2,3-triazole Dihydrochloride (30)—White solid (0.62 g, 58%); mp > 307 °C (dec). ^{1}H NMR (DMSO-d_6) δ 12.08 (br s, 1H), 11.09 (br s, 2H), 10.67 (br s, 2H), 9.24 (s, 1H), 8.81 (s, 1H), 8.36 (s, 4H), 7.99 (d, *J* = 8.8 Hz, 1H), 7.42 (d, *J* = 8.8 Hz, 1H), 4.04 (s, 4H), 3.99 (s, 4H). HPLC (method A) t_R 7.62 min (100 area %). *M/Z* 374.7 (MH$^+$ of free base). Anal. ($C_{20}H_{19}N_7O{\cdot}2HCl{\cdot}1.5H_2O$) C, H, N, Cl.

1-(3-Amidinophenyl)-4-(4-amidinophenyl)-1*H*-1,2,3-triazole Dihydrochloride (31)—White solid (0.09 g, 16%); mp 342–345 °C (dec). ^{1}H NMR (DMSO-d_6) δ 9.87 (s, 1H), 9.75 (br s, 2H), 9.50 (br s, 2H), 9.46 (br s, 2H), 9.28 (br s, 2H), 8.61 (s, 1H), 8.35 (d, *J* = 8.2 Hz, 1H), 8.17 (d, *J* = 8.2 Hz, 2H), 8.02 (d, *J* = 8.2 Hz, 2H), 8.01 (d, *J* = 7.7 Hz, 1H), 7.91 (dd, *J* = 8.2 and 7.7 Hz, 1H). HPLC (method A) t_R 5.19 min (100 area %). Anal. ($C_{16}H_{15}N_7{\cdot}2HCl{\cdot}2H_2O$) C, H, N, Cl.

1-(3-*N*-Isopropylamidinophenyl)-4-(4-*N*-isopropylamidinophenyl)-1*H*-1,2,3-triazole Dihydrochloride (32)—White solid (0.11 g, 15%); mp 324–325 °C (dec). ^{1}H NMR (DMSO-d_6) δ 9.99 (d, *J* = 8.2 Hz, 1H), 9.92 (s, 1H), 9.81 (br s, 1H), 9.72 (d, *J* = 7.7 Hz, 1H), 9.58 (br s, 1H), 9.44 (br s, 1H), 9.25 (br s, 1H), 8.49 (s, 1H), 8.32 (d, *J* = 7.7 Hz, 1H), 8.16 (d, *J* = 8.2 Hz, 2H), 7.92 (d, *J* = 8.2 Hz, 2H), 7.90 (d, *J* = 7.7 Hz, 1H), 7.87 (dd, *J*

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= 7.7 and 7.7 Hz, 1H), 4.15 (m, 2H), 1.32 (d, *J* = 6.6 Hz, 6H), 1.30 (d, *J* = 6.6 Hz, 6H). HPLC (method A) t_R 7.63 min (100 area %). Anal. ($C_{22}H_{27}N_7$·2HCl·1.1H_2O) C, H, N, Cl.

1-[3-(4,5-Dihydro-1*H*-imidazol-2-yl)phenyl]-4-[4-(4,5-dihydro-1*H*-imidazol-2-yl)phenyl]-1*H*-1,2,3-triazole Dihydrochloride (33)—Off-white solid (0.10 g, 18%); mp > 275 °C (dec). ^{1}H NMR (DMSO-d_6) δ 11.2 (br s, 1H), 10.9 (br s, 2H), 9.92 (s, 1H), 9.01 (s, 1H), 8.38 (d, *J* = 8.2 Hz, 1H), 8.30 – 8.15 (m, 6H), 7.94 (dd, *J* = 8.2 and 8.2 Hz, 1H), 4.07 (s, 4H), 4.03 (s, 4H). HPLC (method A) t_R 6.86 min (100 area %). Anal. ($C_{20}H_{19}N_7$·2HCl·1.9H_2O) C, H, N, Cl.

1-(5-Amidino-2-methoxyphenyl)-4-(4-amidinophenyl)-1*H*-1,2,3-triazole Dihydrochloride (34)—White solid (0.65 g, 57%); mp > 247 °C (dec). ^{1}H NMR (DMSO-d_6) δ 9.51 (br s, 4H), 9.30 (s, 1H), 9.26 (br s, 4H), 8.31 (d, *J* = 1.8 Hz, 1H), 8.20 (d, *J* = 8.2 Hz, 2H), 8.15 (d, *J* = 8.2 Hz, 1H), 8.00 (d, *J* = 8.2 Hz, 2H), 7.63 (d, *J* = 8.2 Hz, 1H), 4.03 (s, 3H). HPLC (method A) t_R 6.03 min (100 area %). *M/Z* 336.2 (MH^+ of free base). Anal. ($C_{17}H_{17}N_7O$·2HCl·2.2H_2O) C, H, N, Cl.

1-(5-*N*-Isopropylamidino-2-methoxyphenyl)-4-(4-*N*-isopropylamidinophenyl)-1*H*-1,2,3-triazole Dihydrochloride (35)—White solid (0.63 g, 46%); mp > 250 °C (dec). ^{1}H NMR (DMSO-d_6) δ 9.65 (d, *J* = 6.0 Hz, 2H), 9.51 (br s, 2H), 9.29 (s, 1H), 9.12 (br s, 2H), 8.20 (d, *J* = 8.8 Hz, 2H), 8.17 (s, 1H), 8.00 (d, *J* = 7.7 Hz, 1H), 7.87 (d, *J* = 7.8 Hz, 2H), 7.60 (d, *J* = 8.8 Hz, 1H), 4.08 (m, 2H), 4.01 (s, 3H), 1.30 (s, 12H). HPLC (method A) t_R 8.05 min (100 area %). *M/Z* 420.3 (MH^+ of free base). Anal. ($C_{23}H_{29}N_7O$·2HCl·1.5H_2O) C, H, N, Cl.

1-[5-(4,5-Dihydro-1*H*-imidazol-2-yl)-2-methoxyphenyl]-4-[4-(4,5-dihydro-1*H*-imidazol-2-yl)phenyl]-1*H*-1,2,3-triazole Dihydrochloride (36)—White solid (0.76 g, 59%); mp 250–258 °C. ^{1}H NMR (DMSO-d_6/CD_3OD) δ 9.19 (s, 1H), 8.47 (d, *J* = 2.4 Hz, 1H), 8.26 (d, *J* = 8.2 Hz, 2H), 8.19 (d, *J* = 8.2 Hz, 1H), 8.08 (d, *J* = 8.2 Hz, 2H), 7.66 (d, *J* = 8.2 Hz, 1H), 4.08 (s, 8H), 4.05 (s, 3H). HPLC (method A) t_R 7.42 min (100 area %). *M/Z* 388.2 (MH^+ of free base). Anal. ($C_{21}H_{21}N_7O$·2.4HCl·3.1H_2O) C, H, N, Cl.

4-(4-Amidino-2-methoxyphenyl)-1-(3-amidinophenyl)-1*H*-1,2,3-triazole Dihydrochloride (37)—White solid (0.35 g, 44%); mp > 238 °C (dec). ^{1}H NMR (DMSO-d_6) δ 9.78 (br s, 2H), 9.56 (br s, 2H), 9.44 (s, 1H), 9.40 (br s, 2H), 9.26 (br s, 2H), 8.59 (m, 1H), 8.42 (d, *J* = 8.2 Hz, 1H), 8.40 (d, *J* = 7.9 Hz, 1H), 7.99 (d, *J* = 7.9 Hz, 1H), 7.90 (dd, *J* = 7.9 and 7.9 Hz, 1H), 7.69 (d, *J* = 1.4 Hz, 1H), 7.62 (dd, J = 8.2 and 1.4 Hz, 1H), 4.15 (s, 3H). HPLC (method A) t_R 6.41 min (100 area %). *M/Z* 336.7 (MH^+ of free base). Anal. ($C_{17}H_{17}N_7O$·2.2HCl·1.4H_2O) C, H, N, Cl.

4-(4-*N*-Isopropylamidino-2-methoxyphenyl)-1-(3-*N*-isopropylamidinophenyl)-1*H*-1,2,3-triazole Dihydrochloride (38)—White solid (0.64 g, 68%); mp > 240 °C (dec). ^{1}H NMR (DMSO-d_6) δ 10.04 (d, *J* = 7.2 Hz, 1H), 9.85 (br s, 1H), 9.74 (d, *J* = 7.4 Hz, 1H), 9.62 (br s, 1H), 9.48 (s, 1H), 9.36 (br s, 1H), 9.23 (br s, 1H), 8.48 (s, 1H), 8.41 (d, *J* = 8.1 Hz, 1H), 8.36 (d, *J* = 7.4 Hz, 1H), 7.89 (d, *J* = 7.6 Hz, 1H), 7.86 (dd, *J* = 7.6 and 7.4 Hz, 1H), 7.57 (s, 1H), 7.49 (d, *J* = 8.1 Hz, 1H), 4.15 (s, 3H), 4.13 (m, 2H), 1.33 (d, *J* = 6.2Hz, 6H), 1.31 (d, *J* = 6.2 Hz, 6H). HPLC (method A) t_R 8.26 min (100 area %). *M/Z* 420.7 (MH^+ of free base). Anal. ($C_{23}H_{29}N_7O$·2HCl·2H_2O) C, H, N, Cl.

4-[4-(4,5-Dihydro-1*H*-imidazol-2-yl)-2-methoxyphenyl]-1-[3-(4,5-dihydro-1*H*-imidazol-2-yl)phenyl]-1*H*-1,2,3-triazole Dihydrochloride (39)—White solid (0.82 g,

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94%); mp > 268 °C (dec). ^{1}H NMR (DMSO-d_6) δ 11.31 (br s, 2H), 10.97 (br s, 2H), 9.52 (s, 1H), 9.01 (s, 1H), 8.47 (dd, *J* = 8.2 and 1.6 Hz, 1H), 8.45 (d, *J* = 8.2 Hz, 1H), 8.18 (d, *J* = 8.2 Hz, 1H), 7.98 (br s, 1H), 7.93 (dd, *J* = 8.2 and 8.2 Hz, 1H), 7.80 (dd, *J* = 8.2 and 1.6 Hz, 1H), 4.16 (s, 3H), 4.07 (s, 4H), 4.04 (s, 4H). HPLC (method A) t_R 7.88 min (100 area %). *M/Z* 388.6 (MH^+ of free base). Anal. ($C_{21}H_{21}N_7O{\cdot}2HCl{\cdot}3.5H_2O$) C, H, N, Cl.

1-(5-Amidino-2-hydroxyphenyl)-4-(4-amidinophenyl)-1*H*-1,2,3-triazole Dihydrochloride (40)—Dark-red solid (0.22 g, 50%); mp 240 °C (dec). ^{1}H NMR (DMSO-d_6) δ 12.24 (s, 1H), 9.48 (br s, 2H), 9.38 (br s, 2H), 9.28 (s, 1H), 9.22 (br s, 2H), 9.12 (br s, 2H), 8.25 (s, 1H), 8.22 (d, *J* = 8.2 Hz, 2H), 7.99 (d, *J* = 8.2 Hz, 2H), 7.96 (d, *J* = 8.4 Hz, 1H), 7.48 (d, *J* = 8.4 Hz, 1H). HPLC (method A) t_R 5.13 min (100 area %). *M/Z* 322.7 (MH^+ of free base). Anal. ($C_{16}H_{15}N_7O{\cdot}2.2HCl{\cdot}2.3H_2O$) C, H, N, Cl.

1-(5-*N*-Isopropylamidino-2-hydroxyphenyl)-4-(4-*N*-isopropylamidinophenyl)-1*H*-1,2,3-triazole Dihydrochloride (41)—Dark-red solid (0.25 g, 47%); mp > 235 °C (dec). ^{1}H NMR (DMSO-d_6) δ 12.14 (s, 1H), 9.66 (d, *J* = 7.5 Hz, 1H), 9.56 (d, *J* = 7.6 Hz, 1H), 9.52 (br s, 1H), 9.45 (br s, 1H), 9.28 (s, 1H), 9.17 (br s, 1H), 9.06 (br s, 1H), 8.20 (d, *J* = 8.1 Hz, 2H), 8.10 (s, 1H), 7.88 (d, *J* = 8.1 Hz, 2H), 7.82 (d, *J* = 8.8 Hz, 1H), 7.49 (d, *J* = 8.8 Hz, 1H), 4.08 (m, 2H), 1.31 (d, *J* = 6.4 Hz, 6H), 1.29 (d, *J* = 6.4 Hz, 6H). HPLC (method A) t_R 7.74 min (100 area %). *M/Z* 406.9 (MH^+ of free base). Anal. ($C_{22}H_{27}N_7O{\cdot}2.1HCl{\cdot}2.6H_2O$) C, H, N, Cl.

1-[5-(4,5-Dihydro-1*H*-imidazol-2-yl)-2-hydroxyphenyl]-4-[4-(4,5-dihydro-1*H*-imidazol-2-yl)phenyl]-1*H*-1,2,3-triazole Dihydrochloride (42)—Dark-red solid (0.16 g, 32%); mp 245 °C (dec). ^{1}H NMR (DMSO-d_6) δ 12.51 (s, 1H), 10.87 (s, 2H), 10.72 (s, 2H), 9.31 (s, 1H), 8.47 (s, 1H), 8.26 (d, *J* = 8.3 Hz, 2H), 8.20 (d, *J* = 8.3 Hz, 2H), 8.16 (d, *J* = 8.8 Hz, 1H), 7.54 (d, *J* = 8.8 Hz, 1H), 4.03 (s, 4H), 3.99 (s, 4H). HPLC (method A) t_R 6.98 min (100 area %). *M/Z* 374.7 (MH^+ of free base). Anal. ($C_{20}H_{19}N_7O{\cdot}2.2HCl{\cdot}3.3H_2O$) C, H, N, Cl.

4-(4-Amidino-2-hydroxyphenyl)-1-(3-amidinophenyl)-1*H*-1,2,3-triazole Dihydrochloride (43)—Off-white solid (0.38 g, 46%); mp > 240 °C (dec). ^{1}H NMR (DMSO-d_6) δ 11.36 (s, 1H), 9.72 (br s, 2H), 9.46 (br s, 2H), 9.43 (br s, 2H), 9.29 (s, 1H), 9.22 (s, 2H), 8.49 (s, 1H), 8.38 (d, *J* = 8.0 Hz, 1H), 8.32 (d, *J* = 8.2 Hz, 1H), 7.98 (d, *J* = 8.0 Hz, 1H), 7.86 (dd, *J* = 8.0 and 8.0 Hz, 1H), 7.52 (d, *J* = 1.6 Hz, 1H), 7.36 (dd, *J* = 8.2 and 1.6 Hz, 1H). HPLC (method A) t_R 5.81 min (100 area %). *M/Z* 322.6 (MH^+ of free base). Anal. ($C_{16}H_{15}N_7O{\cdot}2.5HCl{\cdot}0.9H_2O$) C, H, N, Cl.

4-(4-*N*-Isopropylamidino-2-hydroxyphenyl)-1-(3-*N*-isopropylamidinophenyl)-1*H*-1,2,3-triazole Dihydrochloride (44)—Off-white solid (0.42 g, 42%); mp > 245 °C (dec). ^{1}H NMR (DMSO-d_6) δ 11.38 (s, 1H), 9.92 (d, *J* = 7.7 Hz, 1H), 9.74 (br s, 1H), 9.66 (d, *J* = 7.7 Hz, 1H), 9.49 (br s, 1H), 9.39 (br s, 1H), 9.28 (s, 1H), 9.16 (br s, 1H), 8.40 (s, 1H), 8.36 (d, *J* = 7.8 Hz, 1H), 8.31 (d, *J* = 7.8 Hz, 1H), 7.87 (d, *J* = 8.2 Hz, 1H,), 7.82 (dd, *J* = 7.8 and 7.8 Hz, 1H), 7.48 (s, 1H), 7.30 (d, *J* = 8.2 Hz, 1H), 4.11 (m, 2H), 1.32 (d, *J* = 6.3 Hz, 6H), 1.28 (d, *J* = 6.3 Hz, 6H). HPLC (method A) t_R 8.04 min (100 area %). *M/Z* 406.6 (MH^+ of free base). Anal. ($C_{22}H_{27}N_7O{\cdot}2HCl{\cdot}1H_2O{\cdot}0.4EtOH$) C, H, N, Cl.

4-[4-(4,5-Dihydro-1*H*-imidazol-2-yl)-2-hydroxyphenyl]-1-[3-(4,5-dihydro-1*H*-imidazol-2-yl)phenyl]-1*H*-1,2,3-triazole Dihydrochloride (45)—Off-white solid (0.56 g, 60%); mp > 260 °C (dec). ^{1}H NMR (DMSO-d_6) δ 11.46 (br s, 1H), 10.95 (br s, 4H), 9.35 (s, 1H), 8.84 (s, 1H), 8.44 (dd, *J* = 8.2 and 1.2 Hz, 1H), 8.35 (d, *J* = 8.2 Hz, 1H), 8.20

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(d, *J* = 8.2 Hz, 1H), 7.91 (dd, *J* = 8.2 and 8.2 Hz, 1H), 7.62 (d, *J* = 1.2 Hz, 1H), 7.55 (d, *J* = 8.2 Hz, 1H), 4.06 (s, 4H), 4.01 (s, 4H). HPLC (method A) t_R 7.51 min (97.1 area %). *M/Z* 374.9 (MH^+ of free base). Anal. ($C_{20}H_{19}N_7O{\cdot}2HCl{\cdot}2.5H_2O$) C, H, N, Cl.

1,4-Bis(4-amidinophenyl)-1*H*-1,2,3-triazole Dihydrochloride (46)—Off-white solid (0.22 g, 48%); mp 357–359 °C. ^{1}H NMR (DMSO-d_6) δ 9.80 (s, 1H), 9.46 (br s, 6H), 8.26 (d, *J* = 8.8 Hz, 2H), 8.19 (d, *J* = 8.2 Hz, 2H), 8.14 (d, *J* = 8.8 Hz, 2H), 8.01 (d, *J* = 8.2 Hz, 2H). HPLC (method A) t_R 5.46 min (100 area %). Anal. ($C_{16}H_{15}N_7{\cdot}2HCl{\cdot}0.8H_2O$) C, H, N, Cl.

1,4-Bis(4-*N*-isopropylamidinophenyl)-1*H*-1,2,3-triazole Dihydrochloride (47)—White solid (0.09 g, 16%); mp 315–318 °C (dec). ^{1}H NMR (DMSO-d_6) δ 9.82 (d, *J* = 8.1 Hz, 1H), 9.81 (s, 1H), 9.70 (d, *J* = 7.7 Hz, 1H), 9.68 (br s, 1H), 9.56 (br s, 1H), 9.32 (br s, 1H), 9.23 (br s, 1H), 8.24 (d, *J* = 8.8 Hz, 1H), 8.19 (d, *J* = 8.2 Hz, 1H), 8.03 (d, *J* = 8.8 Hz, 1H), 7.91 (d, *J* = 8.2 Hz, 1H), 4.12 (m, 2H), 1.31 (d, *J* = 6.7 Hz, 12H). HPLC (method A) t_R 7.82 min (100 area %). Anal. ($C_{22}H_{27}N_7{\cdot}2HCl{\cdot}1.6H_2O$) C, H, N, Cl.

1,4-Bis(4-(4,5-dihydro-1*H*-imidazol-2-yl)phenyl)-1*H*-1,2,3-triazole Dihydrochloride (48)—Off-white solid (0.32 g, 66%); mp > 360 °C. ^{1}H NMR (DMSO-d_6) δ 10.6 (br s, 4H), 9.73 (s, 1H), 8.27 (m, 4H), 8.22 (d, *J* = 8.8 Hz, 2H), 8.14 (d, *J* = 8.8 Hz, 1H), 4.06 (s, 8H). HPLC (method A) t_R 7.07 min (100 area %). Anal. ($C_{20}H_{19}N_7{\cdot}2HCl{\cdot}0.6H_2O$) C, H, N, Cl.

1-(4-Amidino-2-methoxyphenyl)-4-(4-amidinophenyl)-1*H*-1,2,3-triazole Dihydrochloride (49)—Yellow solid (0.81 g, 66%); mp 315–317 °C (dec). ^{1}H NMR (DMSO-d_6) δ 9.76 (br s, 2H), 9.54 (br s, 2H), 9.49 (br s, 2H), 9.33 (s, 1H), 9.30 (br s, 2H), 8.22 (d, *J* = 8.4 Hz, 2H), 8.01 (d, *J* = 8.4 Hz, 3H), 7.89 (d, *J* = 1.8 Hz, 1H), 7.67 (dd, *J* = 8.4 and 1.7 Hz, 1H), 4.06 (s, 3H). HPLC (method A) t_R 5.91 min (100 area %). *M/Z* 336.1 (MH^+ of free base). Anal. ($C_{17}H_{17}N_7O{\cdot}2.2HCl{\cdot}4.3H_2O$) C, H, N, Cl.

1-(4-*N*-Isopropylamidino-2-methoxyphenyl)-4-(4-*N*-isopropylamidinophenyl)-1*H*-1,2,3-triazole Dihydrochloride (50)—Yellow solid (0.90 g, 61%); mp 245–247 °C (dec). ^{1}H NMR (DMSO-d_6) δ 9.91 (d, *J* = 7.6 Hz, 1H), 9.78 (br s, 1H), 9.69 (d, *J* = 7.4 Hz, 1H), 9.55 (br s, 1H), 9.38 (br s, 1H), 9.31 (s, 1H), 9.20 (br s, 1H), 8.20 (d, *J* = 8.4 Hz, 2H), 7.98 (d, *J* = 8.2 Hz, 1H), 7.88 (d, *J* = 8.4 Hz, 2H), 7.77 (d, *J* = 1.8 Hz, 1H), 7.55 (dd, *J* = 8.2 and 1.8 Hz, 1H), 4.13 (m, 2H), 4.06 (s, 3H), 1.32 (d, *J* = 6.7 Hz, 6H), 1.30 (d, *J* = 6.7 Hz, 6H). HPLC (method A) t_R 8.07 min (100 area %). *M/Z* 420.2 (MH^+ of free base). Anal. ($C_{23}H_{29}N_7O{\cdot}2.5HCl{\cdot}2.2H_2O$) C, H, N, Cl.

1-[4-(4,5-Dihydro-1*H*-imidazol-2-yl)-2-methoxyphenyl]-4-[4-(4,5-dihydro-1*H*-imidazol-2-yl)phenyl]-1*H*-1,2,3-triazole Dihydrochloride (51)—Yellow solid (1.00 g, 73%); mp > 320 °C (dec). ^{1}H NMR (DMSO-d_6) δ 11.11 (br s, 4H), 9.36 (s, 1H), 8.28 (d, *J* = 1.6 Hz, 1H), 8.24 (s, 4H), 8.06 (d, *J* = 8.3 Hz, 1H), 7.90 (dd, *J* = 8.3 and 1.6 Hz, 1H), 4.06 (s, 4H), 4.05 (s, 4H), 4.02 (s, 3H). HPLC (method A) t_R 7.47 min (100 area %). *M/Z* 388.2 (MH^+ of free base). Anal. ($C_{21}H_{21}N_7O{\cdot}2.2HCl{\cdot}2.4H_2O$) C, H, N, Cl.

4-(4-Amidino-2-methoxyphenyl)-1-(4-amidinophenyl)-1*H*-1,2,3-triazole Dihydrochloride (52)—White solid (0.34 g, 59%); mp 301–302 °C (dec). ^{1}H NMR (DMSO-d_6) δ 9.50 (br s, 8H), 9.33 (s, 1H), 8.41 (d, *J* = 8.2 Hz, 1H), 8.36 (d, *J* = 8.8 Hz, 2H), 8.13 (d, *J* = 8.8 Hz, 2H), 7.70 (d, *J* = 1.6 Hz, 1H), 7.62 (dd, *J* = 8.2 and 1.6 Hz, 1H), 4.13 (s, 3H). HPLC (method A) t_R 6.43 min (100 area %). Anal. ($C_{17}H_{17}N_7O{\cdot}2HCl{\cdot}1.8H_2O$) C, H, N, Cl.

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4-(4-*N*-Isopropylamidino-2-methoxyphenyl)-1-(4-*N*-isopropylamidinophenyl)-1*H*-1,2,3-triazole Dihydrochloride (53)—Off-white solid (0.38 g, 54%); mp 267–270 °C (dec). ^{1}H NMR (DMSO-d_6) δ 9.83 (d, J = 7.7 Hz, 1H), 9.77 (d, J = 8.1 Hz, 1H), 9.67 (br s, 1H), 9.65 (br s, 1H), 9.31 (s, 1H), 9.30 (br s, 1H), 9.25 (br s, 1H), 8.39 (d, J = 8.2 Hz, 1H), 8.33 (d, J = 8.8 Hz, 2H), 8.01 (d, J = 8.8 Hz, 2H), 7.59 (br s, 1H), 7.50 (d, J = 8.2 Hz, 1H), 4.14 (s, 3H), 4.13 (m, 2H), 1.32 (d, J = 6.6 Hz, 12H). HPLC (method A) t_R 8.31 min (100 area %). Anal. ($C_{23}H_{29}N_7O{\cdot}2HCl{\cdot}2.7H_2O$) C, H, N, Cl.

4-[4-(4,5-Dihydro-1*H*-imidazol-2-yl)-2-methoxyphenyl]-1-[4-(4,5-dihydro-1*H*-imidazol-2-yl)phenyl]-1*H*-1,2,3-triazole Dihydrochloride (54)—Off-white solid (0.39 g, 59%); mp 320–321 °C (dec). ^{1}H NMR (DMSO-d_6) δ 11.0 (br s, 4H), 9.35 (s, 1H), 8.44 (d, J = 8.2 Hz, 1H), 8.39 (d, J = 8.8 Hz, 2H), 8.35 (d, J = 8.8 Hz, 2H), 8.02 (d, J = 1.1 Hz, 1H), 7.80 (dd, J = 8.2 and 1.1 Hz, 1H), 4.13 (s, 3H), 4.05 (s, 4H), 4.04 (s, 4H). HPLC (method A) t_R 7.93 min (100 area %). Anal. ($C_{21}H_{23}N_7O{\cdot}2HCl{\cdot}1.8H_2O$) C, H, N, Cl.

1-(4-Amidino-2-hydroxyphenyl)-4-(4-amidinophenyl)-1*H*-1,2,3-triazole Dihydrochloride (55)—Off-white solid (0.95 g, 84%); mp > 315 °C (dec). ^{1}H NMR (DMSO-d_6) δ 11.78 (s, 1H), 9.56 (br s, 2H), 9.50 (br s, 2H), 9.32 (br s, 2H), 9.30 (s, 1H), 9.27 (br s, 2H), 8.23 (d, J = 8.2 Hz, 2H), 7.99 (d, J = 8.2 Hz, 2H), 7.93 (d, J = 8.3 Hz, 1H), 7.65 (s, 1H), 7.42 (d, J = 8.3 Hz, 1H). HPLC (method A) t_R 5.43 min (100 area %). *M/Z* 322.2 (MH^+ of free base). Anal. ($C_{16}H_{15}N_7O{\cdot}2HCl{\cdot}1.2H_2O$) C, H, N, Cl.

1-(4-*N*-Isopropylamidino-2-hydroxyphenyl)-4-(4-*N*-isopropylamidinophenyl)-1*H*-1,2,3-triazole Dihydrochloride (56)—White solid (0.53 g, 38%); mp > 258 °C (dec). ^{1}H NMR (DMSO-d_6) δ 11.84 (s, 1H), 9.79 (d, J = 7.7 Hz, 1H), 9.69 (d, J = 6.7 Hz, 1H), 9.61 (br s, 1H), 9.54 (br s, 1H), 9.29 (br s, 2H), 9.21 (br s, 1H), 8.21 (d, J = 7.9 Hz, 2H), 7.91 (d, J = 8.2 Hz, 2H), 7.88 (d, J = 7.9 Hz, 1H), 7.61 (s, 1H), 7.33 (d, J = 8.2 Hz, 1H), 4.11 (m, 2H), 1.30 (d, J = 6.2 Hz, 6H), 1.29 (d, J = 6.2 Hz, 6H). HPLC (method A) t_R 7.87 min (100 area %). *M/Z* 406.3 (MH^+ of free base). Anal. ($C_{22}H_{27}N_7O{\cdot}2HCl{\cdot}1.5H_2O$) C, H, N, Cl.

1-[4-(4,5-Dihydro-1*H*-imidazol-2-yl)-2-hydroxyphenyl]-4-[4-(4,5-dihydro-1*H*-imidazol-2-yl)phenyl]-1*H*-1,2,3-triazole Dihydrochloride (57)—White solid (0.40 g, 31%); mp > 360 °C. ^{1}H NMR (DMSO-d_6) δ 10.72 (br s, 4H), 9.33 (s, 1H), 8.27 (d, J = 8.3 Hz, 2H), 8.14 (d, J = 8.3 Hz, 2H), 8.00 (d, J = 8.3 Hz, 1H), 7.69 (s, 1H), 7.55 (d, J = 8.3 Hz, 1H), 4.03 (s, 8H). HPLC (method A) t_R 7.21 min (100 area %). *M/Z* 374.3 (MH^+ of free base). Anal. ($C_{20}H_{19}N_7O{\cdot}2HCl{\cdot}0.5H_2O$) C, H, N, Cl.

4-(4-Amidino-2-hydroxyphenyl)-1-(4-amidinophenyl)-1*H*-1,2,3-triazole Dihydrochloride (58)—White solid (0.34 g, 42%); mp > 312 °C (dec). ^{1}H NMR (DMSO-d_6) δ 11.33 (s, 1H), 9.62 (br s, 2H), 9.44 (br s, 2H), 9.38 (br s, 2H), 9.28 (s, 1H), 9.22 (br s, 2H), 8.34 (d, J = 8.8 Hz, 2H), 8.32 (d, J = 8.2 Hz, 1H), 8.11 (d, J = 8.8 Hz, 2H), 7.53 (d, J = 1.7 Hz, 1H), 7.38 (dd, J = 8.2 and 1.7 Hz, 1H). HPLC (method A) t_R 5.95 min (100 area %). *M/Z* 322.5 (MH^+ of free base). Anal. ($C_{16}H_{15}N_7O{\cdot}2HCl{\cdot}1.2H_2O$) C, H, N, Cl.

4-(4-*N*-Isopropylamidino-2-hydroxyphenyl)-1-(4-*N*-isopropylamidinophenyl)-1*H*-1,2,3-triazole Dihydrochloride (59)—White solid (0.54 g, 55%); mp > 286 °C (dec). ^{1}H NMR (DMSO-d_6) δ 11.36 (s, 1H), 9.80 (d, J = 8.0 Hz, 1H), 9.66 (d, J = 8.0 Hz, 1H), 9.65 (br s, 1H), 9.49 (br s, 1H), 9.31 (br s, 1H), 9.24 (s, 1H), 9.17 (br s, 1H), 8.31 (d, J = 7.7 Hz, 1H), 8.30 (d, J = 8.8 Hz, 2H), 7.98 (d, J = 8.8 Hz, 2H), 7.48 (d, J = 1.6 Hz, 1H), 7.29 (dd, J = 8.0 and 1.6 Hz, 1H), 4.12 (m, 2H), 1.31 (d, J = 6.7

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Hz, 6H), 1.29 (d, J = 6.7 Hz, 6H). HPLC (method A) t_R 8.18 min (100 area %). *M/Z* 406.7 (MH^+ of free base). Anal. ($C_{22}H_{27}N_7O{\cdot}2HCl{\cdot}1.2H_2O$) C, H, N, Cl.

4-[4-(4,5-Dihydro-1*H*-imidazol-2-yl)-2-hydroxyphenyl]-1-[4-(4,5-dihydro-1*H*-imidazol-2-yl)phenyl]-1*H*-1,2,3-triazole Dihydrochloride (60)—Light-yellow solid (0.52 g, 57%); mp > 345 °C (dec). ^{1}H NMR (DMSO-d_6) δ 11.34 (s, 1H), 10.62 (br s, 4H), 9.27 (s, 1H), 8.36 (d, J = 8.2 Hz, 2H), 8.24 (d, J = 8.2 Hz, 2H), 8.09 (d, J = 1.7 Hz, 1H), 7.54 (dd, J = 8.2 and 1.7 Hz, 1H), 7.52 (d, J = 8.2 Hz, 1H), 4.03 (s, 4H), 4.01 (s, 4H). HPLC (method A) t_R 7.60 min (100 area %). *M/Z* 374.5 (MH^+ of free base). Anal. ($C_{20}H_{19}N_7O{\cdot}2HCl{\cdot}1.5H_2O$) C, H, N, Cl.

3-Azido-4-methoxybenzonitrile (63)—A mixture of 4-hydroxy-3-nitrobenzonitrile (**66**) (14.7 g, 89.4 mmol), K_2CO_3 (14.2 g, 103 mmol), and MeI (14.2 g, 100 mmol) in dry DMF (150 mL) was stirred at ambient temperature. The progress of the reaction was monitored by HPLC. Upon completion, the mixture was poured into iced water and a formed precipitated was collected by filtration, washed with water, and dried to afford 4-methoxy-3-nitrobenzonitrile (**67**) (11.5 g, 73%). ^{1}H NMR (DMSO-d_6) δ 8.49 (d, J = 1.8 Hz, 1H), 8.16 (dd, J = 8.8 and 1.8 Hz, 1H), 7.57 (d, J = 8.8 Hz, 1H), 4.02 (s, 3H). HPLC (method B) t_R 4.26 min (100 area %).

A suspension of **67** (11.0 g, 61.8 mmol) and 10% Pd/C (1.00 g) in MeOH (250 mL) was hydrogenated in a Parr apparatus at 60 psi overnight. The reaction mixture was filtered through a pad of Celite, which was rinsed with MeOH. The filtrate was concentrated and dried under vacuum to yield 3-amino-4-methoxybenzonitrile (**68**) (9.00 g, 98%). ^{1}H NMR (DMSO-d_6) δ 6.98 (d, J = 8.4 Hz, 1H), 6.93 (d, J = 8.4 Hz, 1H), 6.90 (s, 1H), 5.22 (br s, 2H), 3.83 (s, 3H). HPLC (method B) t_R 3.28 min (98.6 area %).

A solution of $NaNO_2$ (4.20 g, 60.0 mmol) in water (30 mL) was added to a solution of **68** (8.50 g, 57.4 mmol) in 10% aqueous HCl (120 mL) at 0–5 °C. The reaction mixture was stirred at this temperature for 30 min and then a solution of NaN_3 (4.55 g, 70.0 mmol) in water (60 mL) was added dropwise. The mixture was stirred for 1 h and a precipitate was filtered off, washed with water, and dried to give **63** as a white solid (9.00 g, 91%); mp 63–65 °C. ^{1}H NMR (DMSO-d_6) δ 7.66 (dd, J = 8.6 and 1.8 Hz, 1H), 7.57 (d, J = 1.8 Hz, 1H), 7.26 (d, J = 8.6 Hz, 1H), 3.93 (s, 3H). HPLC (method B) t_R 5.78 min (96.2 area %). Anal. ($C_8H_6N_4O$) C, H, N.

4-Azido-3-methoxybenzonitrile (64)—A solution of $NaNO_2$ (3.50 g, 50.0 mmol) in water (30 mL) was added to a solution of 4-amino-3-methoxybenzonitrile (**71**) (7.00 g, 47.2 mmol) in 10% aqueous HCl (120 mL) at 0–5 °C. The reaction mixture was stirred at this temperature for 30 min and then a solution of NaN_3 (3.90 g, 60.0 mmol) in water (50 mL) was added dropwise. The mixture was stirred for 1 h and a precipitate was filtered off, washed with water, and dried to give **64** as a white solid (7.75 g, 94%); mp 97–98 °C. ^{1}H NMR (DMSO-d_6) δ 7.56 (d, J = 1.6 Hz, 1H), 7.41 (dd, J = 8.2 and 1.6 Hz, 1H), 7.20 (d, J = 8.2 Hz, 1H), 3.88 (s, 3H). HPLC (method B) t_R 5.77 min (100 area %). Anal. ($C_8H_6N_4O$) C, H, N.

3-Azido-4-hydroxybenzonitrile (65)—A solution of $NaNO_2$ (1.54 g, 22.0 mmol) in water (10 mL) was added to a solution of 3-amino-4-hydroxybenzonitrile (**72**) (2.68 g, 20.0 mmol) in 10% aqueous HCl (40 mL) at 0–5 °C. The reaction mixture was stirred at this temperature for 30 min and then a solution of NaN_3 (1.56 g, 24.0 mmol) in water (20 mL) was added dropwise. The mixture was stirred for 1 h and a precipitate was filtered off, washed with water, and dried to give **65** as a white solid (2.00 g, 63%); mp 108 °C (dec). ^{1}H NMR (DMSO-d_6) δ 11.40 (s, 1H), 7.47 (dd, J = 8.1 and 2.0 Hz, 1H), 7.46 (d, J = 2.0 Hz,

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1H), 6.99 (d, *J* = 8.0 Hz, 1H). HPLC (method B) t_R 4.65 min (100 area %). Anal. ($C_7H_4N_4O$) C, H, N.

4-Amino-3-methoxybenzonitrile[82] (71)—A mixture of 4-bromo-*o*-anisidine[81] (**70**) (18.9 g, 93.5 mmol) and CuCN (10.7 g, 119 mmol) in dry DMF (50 mL) and pyridine (10 mL) was refluxed overnight. The mixture was cooled, diluted with $CHCl_3$, and filtered through a pad of Celite. A filtrate was concentrated and dried under vacuum to give crude, which was purified by column chromatography eluting with $CHCl_3$ to afford **71** (7.50 g, 54%); mp 46–48 °C (No lit.[82] mp). ^{1}H NMR (DMSO-d_6) δ 7.13 (d, *J* = 1.8 Hz, 1H), 7.12 (dd, *J* = 8.4 and 1.8 Hz, 1H), 6.67 (d, *J* = 8.4 Hz, 1H), 5.80 (br s, 2H), 3.81 (s, 3H). HPLC (method B) t_R 3.53 min (100 area %). Anal. ($C_8H_8N_2O$) C, H, N.

3-Amino-4-hydroxybenzonitrile (72)—A suspension of 3-nitro-4-hydroxybenzonitrile **66** (10.0 g, 60.9 mmol) and 10% Pd/C (1.00 g) in MeOH (250 mL) was hydrogenated in a Parr apparatus at 60 psi for 2 h. The reaction mixture was filtered through a pad of Celite, which was rinsed with MeOH. The filtrate was concentrated and dried under vacuum to yield **72** (7.15 g, 88%); mp 153–154 °C. ^{1}H NMR (DMSO-d_6) δ 10.21 (br s, 1H), 8.86 (d, *J* = 2.0 Hz, 1H), 8.83 (dd, *J* = 7.8 and 2.0 Hz, 1H), 6.75 (d, *J* = 7.8 Hz, 1H), 4.99 (br s, 2H). HPLC (method A) t_R 4.95 min (100 area %). Anal. ($C_7H_6N_2O$) C, H, N.

General Procedure for Synthesis of Dinitriles 77–89, 93. 1,4-Bis(3-cyanophenyl)-1*H*-1,2,3-triazole (77)—To a suspension of 3-azidobenzonitrile (**61**) (1.44 g, 10.0 mmol) and 3-ethynylbenzonitrile (**73**) (1.27 g, 10.0 mmol) in a 1:1 mixture of water and *t*-butyl alcohol (40 ml) was added a solution of sodium ascorbate (0.20 g, 1 mmol) in water (5 ml) followed by copper(II) sulfate pentahydrate (0.025 g, 0.1 mmol) in water (1 ml). The mixture was stirred at ambient temperature overnight, at which point HPLC analysis indicated presence of starting materials in the mixture. A solution of sodium ascorbate (0.20 g, 1 mmol) in water (5 ml) was added and the mixture was stirred for 24 hrs at 70 °C to complete the reaction. The reaction mixture was diluted with water, a precipitate was collected by filtration, washed with water (3 × 50 mL), and dried under vacuum to give crude (2.60 g, 96%), which was recrystallized to afford **77** as a light-yellow solid; mp 218–219 °C (EtOAc/EtOH). ^{1}H NMR (DMSO-d_6) δ 9.56 (s, 1H), 8.45 (dd, *J* = 1.6 and 1.6 Hz, 1H), 8.32 (dd, *J* = 1.1 and 1.1 Hz, 1H), 8.29 (dd, *J* = 8.2 and 1.6 Hz, 1H), 8.26 (dd, *J* = 8.2 and 1.6 Hz, 1H), 8.02 (dd, *J* = 7.7 and 1.1 Hz, 1H), 7.89 (dd, *J* = 7.7 and 1.1 Hz, 1H), 7.87 (dd, *J* = 7.7 and 7.7 Hz, 1H), 7.75 (dd, *J* = 8.2 and 8.2 Hz, 1H). HPLC (method B) t_R 6.56 min (100 area %). Anal. ($C_{16}H_9N_5$) C, H, N.

1-(4-Cyanophenyl)-4-(3-cyanophenyl)-1*H*-1,2,3-triazole (78)—Following the procedure described above for **77**, **78** was prepared from 4-azidobenzonitrile (**62**) (1.44 g, 10.0 mmol) and **73** (1.27 g, 10.0 mmol). Off-white solid (2.47 g, 91%); mp 217–219 °C (EtOAc). ^{1}H NMR (DMSO-d_6) δ 9.61 (s, 1H), 8.34 (dd, *J* = 1.6 and 1.6 Hz, 1H), 8.27 (dd, *J* = 8.2 and 1.6 Hz, 1H), 8.17 (s, 4H), 7.88 (dd, *J* = 8.2 and 1.6 Hz, 1H), 7.75 (dd, *J* = 8.2 and 8.2 Hz, 1H). HPLC (method B) t_R 6.59 min (100 area %). Anal. ($C_{16}H_9N_5$) C, H, N.

1-(3-Cyanophenyl)-4-(4-cyanophenyl)-1*H*-1,2,3-triazole (79)—Following the procedure described above for **77**, **79** was prepared from **61** (1.44 g, 10.0 mmol) and 4-ethynylbenzonitrile (**74**) (1.27 g, 10.0 mmol). Off-white solid (2.47 g, 91%); mp 223–224 °C (EtOH/DMF). ^{1}H NMR (DMSO-d_6) δ 9.61 (s, 1H), 8.47 (dd, *J* = 1.1 and 1.1 Hz, 1H), 8.34 (dd, *J* = 8.2 and 1.1 Hz, 1H), 8.11 (d, *J* = 8.2 Hz, 2H), 8.03 (dd, *J* = 8.2 and 1.1 Hz, 1H), 8.01 (d, *J* = 8.2 Hz, 2H), 7.87 (dd, *J* = 8.2 and 8.2 Hz, 1H). HPLC (method B) t_R 6.57 min (100 area %). Anal. ($C_{16}H_9N_5$) C, H, N.

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1,4-Bis(4-cyanophenyl)-1*H*-1,2,3-triazole (80)—Following the procedure described above for **77**, **80** was prepared from **62** (1.44 g, 10.0 mmol) and **74** (1.27 g, 10.0 mmol). Orange solid (2.38 g, 88%); mp 261–263 °C (DMF). ^{1}H NMR (DMSO-d_6) δ 9.66 (s, 1H), 8.18 (s, 4H), 8.12 (d, J = 8.8 Hz, 2H), 8.01 (d, J = 8.8 Hz, 2H). HPLC (method B) t_R 6.60 min (100 area %). Anal. ($C_{16}H_9N_5$) C, H, N.

1-(5-Cyano-2-methoxyphenyl)-4-(3-cyanophenyl)-1*H*-1,2,3-triazole (81)— Following the procedure described above for **77**, **81** was prepared from **63** (1.74 g, 10.0 mmol) and **73** (1.27 g, 10.0 mmol) in a mixture of DMSO (45 mL) and water (15 mL). White solid (2.81 g, 93%); mp 228–230 °C (EtOH/DMF). ^{1}H NMR (DMSO-d_6) δ 9.18 (s, 1H), 8.40 (s, 1H), 8.31 (d, J = 7.7 Hz, 1H), 8.27 (d, J = 1.8 Hz, 1H), 8.10 (dd, J = 8.8 and 1.8 Hz, 1H), 7.86 (d, J = 7.7 Hz, 1H), 7.73 (dd, J = 7.7 and 7.7 Hz, 1H), 7.57 (d, J = 8.8 Hz, 1H), 4.00 (s, 3H). HPLC (method B) t_R 6.52 min (100 area %). Anal. ($C_{17}H_{11}N_5O$) C, H, N.

4-(5-Cyano-2-methoxyphenyl)-1-(3-cyanophenyl)-1H-1,2,3-triazole (82)— Following the procedure described above for **77**, **82** was prepared from **61** (2.88 g, 20.0 mmol) and 3-ethynyl-4-methoxybenzonitrile (**75**) (3.14 g, 20.0 mmol) in a mixture of DMSO (90 mL) and water (20 mL). White solid (4.90 g, 81%); mp 280–281 °C (Py). ^{1}H NMR (DMSO-d_6) δ 9.23 (s, 1H), 8.59 (s, 1H), 8.52 (d, J = 1.5 Hz, 1H), 8.41 (d, J = 7.8 Hz, 1H), 8.00 (d, J = 7.8 Hz, 1H), 7.90 (dd, J = 8.8 and 1.5 Hz, 1H), 7.88 (dd, J = 7.8 and 7.8 Hz, 1H), 7.40 (d, J = 8.8 Hz, 1H), 4.09 (s, 3H). HPLC (method B) t_R 7.04 min (100 area %). Anal. ($C_{17}H_{11}N_5O$) C, H, N.

1-(4-Cyano-2-methoxyphenyl)-4-(3-cyanophenyl)-1*H*-1,2,3-triazole (83)— Following the procedure described above for **77**, **83** was prepared from **64** (3.70 g, 21.0 mmol) and **73** (2.70 g, 21.0 mmol) in a mixture of DMSO (70 mL) and water (30 mL). Dark-yellow solid (6.30 g, 99%); mp 228–230 °C (EtOH/DMF). ^{1}H NMR (DMSO-d_6) δ 9.25 (s, 1H), 8.42 (s, 1H), 8.33 (dd, J = 8.1 and 1.3 Hz, 1H), 7.98 (d, J = 8.1 Hz, 1H), 7.93 (d, J = 1.3 Hz, 1H), 7.86 (dd, J = 7.8 and 1.3 Hz, 1H), 7.73 (d, J = 7.8 Hz, 1H), 7.69 (dd, J = 8.1 and 1.3 Hz, 1H), 4.00 (s, 3H). HPLC (method B) t_R 6.74 min (100 area %). Anal. ($C_{17}H_{11}N_5O{\cdot}0.2EtOH{\cdot}0.1H_2O$) C, H, N.

4-(5-Cyano-2-methoxyphenyl)-1-(4-cyanophenyl)-1*H*-1,2,3-triazole (84)— Following the procedure described above for **77**, **84** was prepared from **62** (1.44 g, 10.0 mmol) and **75** (1.60 g, 11.0 mmol). White solid (2.39 g, 79%); mp 288–289 °C (EtOH/DMF). ^{1}H NMR (DMSO-d_6) δ 9.24 (s, 1H), 8.51 (d, J = 1.6 Hz, 1H), 8.29 (d, J = 8.8 Hz, 2H), 8.14 (d, J = 8.8 Hz, 2H), 7.90 (dd, J = 8.8 and 1.6 Hz, 1H), 7.39 (d, J = 8.8 Hz, 1H), 4.08 (s, 3H). HPLC (method B) t_R 6.98 min (100 area %). Anal. ($C_{17}H_{11}N_5O$) C, H, N.

1-(5-Cyano-2-methoxyphenyl)-4-(4-cyanophenyl)-1*H*-1,2,3-triazole (85)— Following the procedure described above for **77**, **85** was prepared from **63** (1.74 g, 10.0 mmol) and **74** (1.27 g, 10.0 mmol) in a mixture of DMSO (45 mL) and water (15 mL). Off-white solid (2.95 g, 98%); mp 235–237 °C (EtOH/DMF). ^{1}H NMR (DMSO-d_6) δ 9.22 (s, 1H), 8.27 (d, J = 1.8 Hz, 1H), 8.14 (d, J = 8.3 Hz, 2H), 8.10 (dd, J = 8.7 and 1.8 Hz, 1H), 7.99 (d, J = 8.3 Hz, 2H), 7.56 (d, J = 8.7 Hz, 1H), 3.99 (s, 3H). HPLC (method B) t_R 6.51 min (100 area %). Anal. ($C_{17}H_{11}N_5O$) C, H, N.

4-(4-Cyano-2-methoxyphenyl)-1-(3-cyanophenyl)-1*H*-1,2,3-triazole (86)— Following the procedure described above for **77**, **86** was prepared from **61** (2.88 g, 20.0 mmol) and 4-ethynyl-3-methoxybenzonitrile (**76**) (3.14 g, 20.0 mmol) in a mixture of DMSO (90 mL) and water (20 mL). Off-white solid (5.87 g, 97%); mp 236–237 °C (Py). ^{1}H NMR (DMSO-d_6) δ 9.27 (s, 1H), 8.59 (s, 1H), 8.40 (d, J = 8.0 Hz, 1H), 8.37 (d, J = 8.0 Hz,

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1H), 8.00 (d, *J* = 8.0 Hz, 1H), 7.85 (dd, *J* = 8.0 and 8.0 Hz, 1H), 7.69 (s, 1H), 7.58 (d, *J* = 8.0 Hz, 1H), 4.07 (s, 3H). HPLC (method B) t_R 7.24 min (100 area %). Anal. ($C_{17}H_{11}N_5O$) C, H, N.

1-(4-Cyano-2-methoxyphenyl)-4-(4-cyanophenyl)-1*H*-1,2,3-triazole (87)— Following the procedure described above for **77**, **87** was prepared from **64** (3.70 g, 21.0 mmol) and **74** (2.70 g, 21.0 mmol) in a mixture of DMSO (70 mL) and water (30 mL). Yellow solid (6.15 g, 97%); mp 240–242 °C (EtOH/DMF). ^{1}H NMR (DMSO-d_6) δ 9.28 (s, 1H), 8.16 (d, *J* = 8.2 Hz, 2H), 7.99 (d, *J* = 8.2 Hz, 3H), 7.93 (d, *J* = 1.4 Hz, 1H), 7.69 (dd, *J* = 8.2 and 1.4 Hz, 1H), 3.99 (s, 3H). HPLC (method B) t_R 6.75 min (100 area %). Anal. ($C_{17}H_{11}N_5O{\cdot}0.3H_2O$) C, H, N.

4-(4-Cyano-2-methoxyphenyl)-1-(4-cyanophenyl)-1*H*-1,2,3-triazole (88)— Following the procedure described above for **77**, **88** was prepared from **62** (3.50 g, 24.3 mmol) and **76** (4.40 g, 28.0 mmol). White solid (7.03 g, 96%); mp 286–287 °C (DMF). ^{1}H NMR (DMSO-d_6) δ 9.29 (s, 1H), 8.37 (d, *J* = 8.2 Hz, 1H), 8.29 (d, *J* = 8.8 Hz, 2H), 8.14 (d, *J* = 8.8 Hz, 2H), 7.69 (d, *J* = 1.6 Hz, 1H), 7.77 (dd, *J* = 8.2 and 1.6 Hz, 1H), 4.06 (s, 3H). HPLC (method B) t_R 7.20 min (100 area %). Anal. ($C_{17}H_{11}N_5O$) C, H, N.

1-(5-Cyano-2-hydroxyphenyl)-4-(3-cyanophenyl)-1*H*-1,2,3-triazole (89)— Following the procedure described above for **77**, **89** was prepared from **65** (1.60 g, 10.0 mmol) and **73** (1.27 g, 10.0 mmol) in a mixture of DMSO (45 mL) and water (15 mL). Purple solid (2.06 g, 72%); mp 285–287 °C (EtOH). ^{1}H NMR (DMSO-d_6) δ 11.98 (s, 1H), 9.17 (s, 1H), 8.41 (s, 1H), 8.31 (d, *J* = 7.7 Hz, 1H), 8.20 (d, *J* = 1.8 Hz, 1H), 7.92–7.80 (m, 2H), 7.72 (dd, *J* = 7.7 and 7.7 Hz, 1H), 7.30 (d, *J* = 8.2 Hz, 1H). HPLC (method B) t_R 6.33 min (100 area %). Anal. ($C_{16}H_9N_5O{\cdot}0.2EtOH$) C, H, N.

General Procedure for Synthesis of Dinitriles 90, 92, 94, 96. 4-(5-Cyano-2-hydroxyphenyl)-1-(3-cyanophenyl)-1H-1,2,3-triazole (90)—To a suspension of **82** (2.55 g, 8.46 mmol) in dry CH_2Cl_2 (120 mL) at 0 °C was added 1M solution of BBr_3 in CH_2Cl_2 (12 mL, 12 mmol). The mixture was stirred at 0 °C for 1h and then at ambient temperature. The progress of the reaction was monitored by HPLC. Upon completion, the reaction mixture was cooled in the ice bath and EtOH (30 mL) was added dropwise. A precipitate was separated, washed with EtOH, and dried under vacuum to give crude (2.40 g, 98%), which was recrystallized to afford **90**. Off-white solid; mp 291 °C (dec) (EtOH/DMF). ^{1}H NMR (DMSO-d_6) δ 11.56 (s, 1H), 9.17 (s, 1H), 8.57 (s, 1H), 8.43 (d, *J* = 2.1 Hz, 1H), 8.38 (d, *J* = 7.7 Hz, 1H), 7.98 (d, *J* = 7.7 Hz, 1H), 7.82 (dd, *J* = 7.7 and 7.7 Hz, 1H), 7.69 (dd, *J* = 8.5 and 2.1 Hz, 1H), 7.18 (d, *J* = 8.5 Hz, 1H). HPLC (method B) t_R 6.63 min (100 area %). Anal. ($C_{16}H_9N_5O$) C, H, N.

General Procedure for Synthesis of Dinitriles 91, 95

1-(4-Cyano-2-hydroxyphenyl)-4-(3-cyanophenyl)-1*H*-1,2,3-triazole (91): To molten pyridine hydrochloride (24 g) was added **83** (4.00 g, 13.3 mmol), and the reaction mixture was kept at 160–180 °C for 3 h, cooled to 80–90 °C and diluted with water (100 mL). A formed precipitate, was separated, washed with water, and dried to give **91** as a grey solid (3.40 g, 89%); mp 284–285 °C (EtOH). ^{1}H NMR (DMSO-d_6) δ 11.62 (s, 1H), 9.24 (s, 1H), 8.43 (s, 1H), 8.33 (d, *J* = 8.0 Hz, 1H), 7.92 (d, *J* = 8.8 Hz, 1H), 7.86 (dd, *J* = 7.7 and 1.6 Hz, 1H), 7.72 (dd, *J* = 7.7 and 7.7 Hz, 1H), 7.52 (dd, *J* = 8.8 and 1.6 Hz, 1H), 7.51 (s, 1H). HPLC (method B) t_R 6.68 min (100 area %). Anal. ($C_{16}H_9N_5O_2{\cdot}0.3EtOH{\cdot}0.3H_2O$) C, H, N.

4-(5-Cyano-2-hydroxyphenyl)-1-(4-cyanophenyl)-1*H*-1,2,3-triazole (92)— Following the procedure described above for **90**, **92** was prepared from **84** (3.50 g, 11.6

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mmol) and 1M BBr_3 in CH_2Cl_2 (15 mL). Off-white solid (2.98 g, 89%); mp 282 °C (dec) (EtOH/DMF). ^{1}H NMR (DMSO-d_6) δ 11.59 (s, 1H), 9.17 (s, 1H), 8.43 (d, J = 2.2 Hz, 1H), 8.26 (d, J = 8.9 Hz, 2H), 8.12 (d, J = 8.9 Hz, 2H), 7.70 (dd, J = 8.5 and 2.2 Hz, 1H), 7.17 (d, J = 8.5 Hz, 1H). HPLC (method B) t_R 6.65 min (100 area %). Anal. ($C_{16}H_9N_5O{\cdot}0.3H_2O$) C, H, N.

1-(5-Cyano-2-hydroxyphenyl)-4-(4-cyanophenyl)-1*H*-1,2,3-triazole (93)— Following the procedure described above for **77**, **93** was prepared from **65** (1.60 g, 10.0 mmol) and **74** (1.27 g, 10.0 mmol) in a mixture of DMSO (45 mL) and water (15 mL). Brown solid (1.30 g, 45%); mp 285–287 °C (EtOH). ^{1}H NMR (DMSO-d_6) δ 11.98 (s, 1H), 9.21 (s, 1H), 8.20 (s, 1H), 8.15 (d, J = 8.0 Hz, 2H), 7.98 (d, J = 8.0 Hz, 2H), 7.88 (d, J = 7.7 Hz, 1H), 7.29 (d, J = 7.7 Hz, 1H). HPLC (method B) t_R 6.34 min (100 area %). Anal. ($C_{16}H_9N_5O{\cdot}0.2DMF{\cdot}0.3H_2O$) C, H, N.

4-(4-Cyano-2-hydroxyphenyl)-1-(3-cyanophenyl)-1*H*-1,2,3-triazole (94)— Following the procedure described above for **90**, **94** was prepared from **86** (2.38 g, 7.90 mmol) and 1M BBr_3 in CH_2Cl_2 (11 mL). White solid (2.20 g, 97%); mp 263 °C (dec) (MeCN). ^{1}H NMR (DMSO-d_6) δ 11.17 (s, 1H), 9.24 (s, 1H), 8.59 (s, 1H), 8.39 (dd, J = 7.8 and 2.2 Hz, 1H), 8.29 (d, J = 8.0 Hz, 1H), 7.99 (d, J = 7.8 Hz, 1H), 7.83 (dd, J = 7.8 and 7.8 Hz, 1H), 7.42 (dd, J = 8.0 and 1.5 Hz, 1H), 7.35 (d, J = 1.5 Hz, 1H). HPLC (method B) t_R 6.90 min (100 area %). Anal. ($C_{16}H_9N_5O$) C, H, N.

1-(4-Cyano-2-hydroxyphenyl)-4-(4-cyanophenyl)-1*H*-1,2,3-triazole (95)— Following the procedure described above for **91**, **95** was prepared from **87** (2.00 g, 6.50 mmol) as an off-white solid (1.70, 89%); mp 276–278 °C (EtOH/DMF). ^{1}H NMR (DMSO-d_6) δ 11.64 (s, 1H), 9.28 (s, 1H), 8.17 (d, J = 8.1 Hz, 2H), 7.98 (d, J = 8.1 Hz, 2H), 7.93 (d, J = 8.2 Hz, 1H), 7.52 (d, J = 8.2 Hz, 1H), 7.50 (s, 1H). HPLC (method B) t_R 6.68 min (100 area %). Anal. ($C_{16}H_9N_5O_2{\cdot}0.2EtOH{\cdot}0.4H_2O$) C, H, N.

4-(4-Cyano-2-hydroxyphenyl)-1-(4-cyanophenyl)-1*H*-1,2,3-triazole (96)— Following the procedure described above for **90**, **96** was prepared from **88** (5.18 g, 17.2 mmol) and 1M BBr_3 in CH_2Cl_2 (24 mL). White solid (4.90 g, 99%); mp 293 °C (dec) (DMF). ^{1}H NMR (DMSO-d_6) δ 11.19 (s, 1H), 9.23 (s, 1H), 8.29 (d, J = 8.0 Hz, 1H), 8.26 (d, J = 8.8 Hz, 2H), 8.12 (d, J = 8.8 Hz, 2H), 7.41 (dd, J = 8.0 and 1.5 Hz, 1H), 7.35 (d, J = 1.5 Hz, 1H). HPLC (method B) t_R 6.92 min (100 area %). Anal. ($C_{16}H_9N_5O$) C, H, N.

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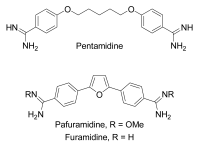

Figure 1.
Structures of pentamidine, pafuramidine, and furamidine.

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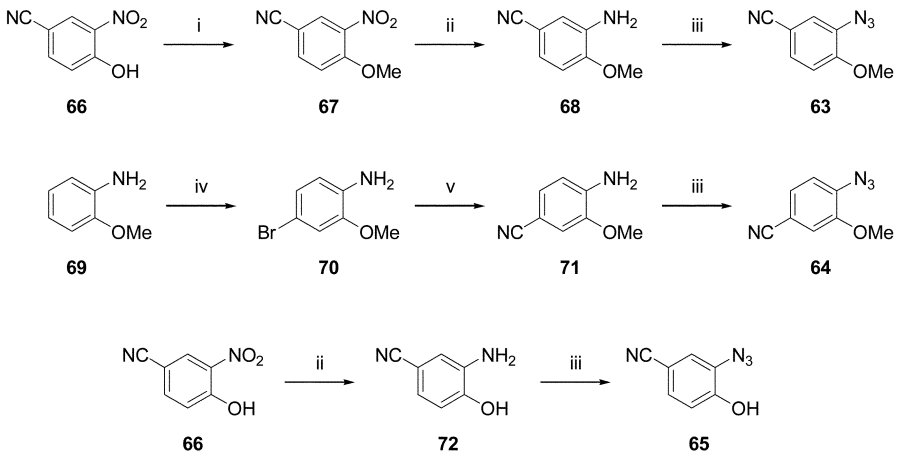
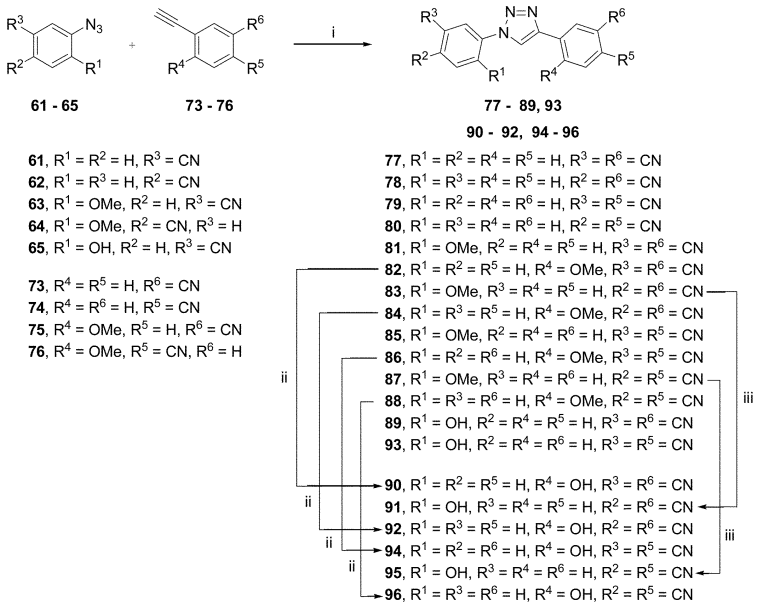


Scheme 1a.

[a] Reagents and conditions: (i) H_2, 50 psi, 10% Pd/C, MeOH; (ii) MeI, K_2CO_3, DMF, ambient temp; (iii) $NaNO_2$, 10% aq HCl, 0–5 °C, 1 h, then NaN_3, H_2O, 0–5 °C, 1 h; (iv) Br_2, AcOH; (v) CuCN, DMF–Py (5:1), reflux.



Scheme 2a.

[a] Reagents and conditions: (i) Sodium ascorbate, $CuSO_4 \cdot 5H_2O$, DMF–H_2O (9:1) or *t*-BuOH–H_2O (1:1), 24–48 h; (ii) BBr_3, CH_2Cl_2, 0 °C–ambient temp; (iii) pyridine hydrochloride, 150–160 °C, 3 h.

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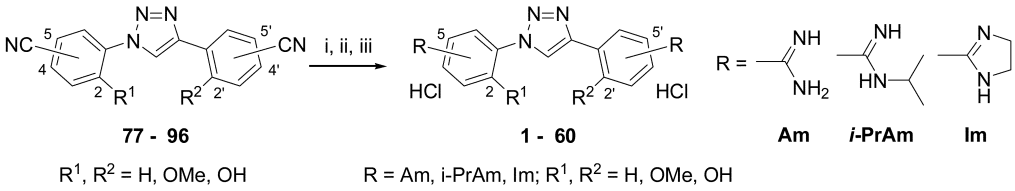

Scheme 3a.

[a] Reagents and conditions: (i) HCl gas, 1,4-Dioxane–EtOH (3:1); (ii) appropriate amine, EtOH, ambient temp; (iii) 3M HCl, EtOH.

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Table 1

Cytotoxicity and in Vitro Antiprotozoal Activity of Dications **1–15**.

1 - 15

R = Am, *i*-PrAm, Im; R^1, R^2 = H, OMe, OH

compd	R	R^1	R^2	Cytotoxicity	*T. brucei rhodesiense*[g]		*P. falciparum*[i]		*L. donovani*[k]	
				IC_{50} (μM)	IC_{50} (μM)	SI_T[h]	IC_{50} (μM)	SI_P[j]	IC_{50} (μM)	SI_L[l]
1	Am	H	H	219	0.083	2639	0.021	10429	48	5
2	*i*-PrAm	H	H	>184	14.8	>12	0.118	>1559	>100	ND[m]
3	Im	H	H	>197	14.1	>14	0.365	>540	>100	ND
4	Am	OMe	H	>224	0.047	>4766	0.028	>8000	>100	ND
5	*i*-PrAm	OMe	H	131	10.1	13	0.125	1048	>100	ND
6	Im	OMe	H	>172	7.83	>22	0.030	>5733	>100	ND
7	Am	H	OMe	>200	1.65	>121	0.024	>8333	>100	ND
8	*i*-PrAm	H	OMe	>176	1.25	>141	0.106	>1660	>100	ND
9	Im	H	OMe	>176	0.006	>29333	0.014	>12571	4.9	>36
10	Am	OH	H	>209	49.6	>4	8.93	>23	>100	ND
11	*i*-PrAm	OH	H	>167	13.9	>12	0.739	>226	>100	ND
12	Im	OH	H	>174	102	>2	0.325	>535	>100	ND
13	Am	H	OH	>136	0.174	>782	0.036	>3778	20	>7
14	*i*-PrAm	H	OH	>170	10.9	>16	0.229	>742	>100	ND
15	Im	H	OH	>183	11.7	>16	0.052	>3519	>100	ND
PMD[a]				46.6	0.003	15533	0.058	803	1.8	25
MLSP[b]				7.78	0.004	1945				
CQ[c]				117			0.124	944		
ATMS[d]				450			0.006	75000		

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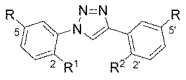
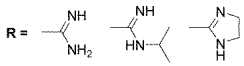


1 - 15

R = Am, *i*-PrAm, Im; R^1, R^2 = H, OMe, OH



Am ***i*-PrAm** **Im**

compd	R	R^1	R^2	Cytotoxicity	*T. brucei rhodesiense*[g]		*P. falciparum*[i]		*L. donovani*[k]	
				IC_{50} (μM)	IC_{50} (μM)	SI_T[h]	IC_{50} (μM)	SI_P[j]	IC_{50} (μM)	SI_L[l]
PPT[e]				0.020						

[a]PMD, Pentamidine.

[b]MLSP, melarsoprol

[c]CQ, chloroquine.

[d]ATMS, artemisinin.

[e]PPT, podophyllotoxin.

[f]Cytotoxicity (L6 rat myoblast cells). Average of duplicate determinations. Maximum test concentration was 90 μg/mL. Based on the molecular weight of tested compounds, different > μM values were obtained from the conversion of 90 μg/mL to the μM scale.

[g]*Trypanosoma brucei rhodesiense* (STIB900). Average of duplicate determinations.

[h]Selectivity index for *T. brucei rhodesiense* (SI_T), expressed as the ratio [IC_{50} (L6)/IC_{50} (*T. brucei rhodesiense*)].

[i]*Plasmodium falciparum* (K1, resistant to chloroquine). Average of duplicate determinations.

[j]Selectivity index for *P. falciparum* (SI_P), expressed as the ratio [IC_{50} (L6)/IC_{50} (*P. falciparum*)].

[k]*Leishmania donovani* (MHOM/SD/62/1S-$CL2_D$) axenic amastigotes. Average of duplicate determinations.

[l]Selectivity index for *L. donovani* (SI_L), expressed as the ratio [IC_{50} (L6)/IC_{50} (*L. donovani*)].

[m]ND, not determined.

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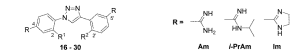
Table 2

Cytotoxicity and in Vitro Antiprotozoal Activity of Dications **16**–**30**.



compd	R	R^1	R^2	Cytotoxicity	*T. brucei rhodesiense*[g]		*P. falciparum*[i]		*L. donovani*[k]	
				IC_{50} (μM)	IC_{50} (μM)	SI_T[h]	IC_{50} (μM)	SI_P[j]	IC_{50} (μM)	SI_L[l]
16	Am	H	H	8.60	0.008	1075	0.003	2867	12	<1
17	*i*-PrAm	H	H	>190	0.539	>353	0.038	>5000	>100	ND[m]
18	Im	H	H	127	0.200	635	0.084	1512	>100	ND
19	Am	OMe	H	>192	0.011	>17455	0.025	>7680	33	>6
20	*i*-PrAm	OMe	H	>159	1.96	>81	0.055	>2891	>100	ND
21	Im	OMe	H	>173	1.87	>93	0.058	>2983	>100	ND
22	Am	H	OMe	>198	0.008	>24750	0.007	>28286	4.3	>46
23	*i*-PrAm	H	OMe	>177	0.077	>2299	0.026	>6808	>100	ND
24	Im	H	OMe	>188	0.021	>8952	0.010	>18800	>100	ND
25	Am	OH	H	>209	0.175	>1194	0.175	>1194	>100	ND
26	*i*-PrAm	OH	H	>187	18.7	>10	1.19	>157	>100	ND
27	Im	OH	H	>187	11.1	>17	0.159	>1176	>100	ND
28	Am	H	OH	>219	0.006	>36500	0.022	>9955	>50	ND
29	*i*-PrAm	H	OH	>179	0.116	>1543	0.028	>6393	>100	ND
30	Im	H	OH	>190	0.043	>4419	0.009	>21111	43	>4
PMD[a]				46.6	0.003	15533	0.058	803	1.8	25
MLSP[b]				7.78	0.004	1945				
CQ[c]				117			0.124	944		
ATMS[d]				450			0.006	75000		
PPT[e]				0.020						

[a]PMD, Pentamidine.

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[b] MLSP, melarsoprol.

[c] CQ, chloroquine.

[d] ATMS, artemisinin.

[e] PPT, podophyllotoxin.

[f] Cytotoxicity (L6 rat myoblast cells). Average of duplicate determinations. Maximum test concentration was 90 μg/mL. Based on the molecular weight of tested compounds, different > μM values were obtained from the conversion of 90 μg/mL to the μM scale.

[g] *Trypanosoma brucei rhodesiense* (STIB900). Average of duplicate determinations.

[h] Selectivity index for *T. brucei rhodesiense* (SI_T), expressed as the ratio [IC_{50} (L6)/IC_{50} (*T. brucei rhodesiense*)].

[i] *Plasmodium falciparum* (K1, resistant to chloroquine). Average of duplicate determinations.

[j] Selectivity index for *P. falciparum* (SI_P), expressed as the ratio [IC_{50} (L6)/IC_{50} (*P. falciparum*)].

[k] *Leishmania donovani* (MHOM/SD/62/1S-$CL2_D$) axenic amastigotes. Average of duplicate determinations.

[l] Selectivity index for *L. donovani* (SI_L), expressed as the ratio [IC_{50} (L6)/IC_{50} (*L. donovani*)].

[m] ND, not determined.

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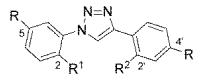
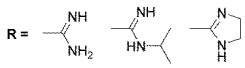
Table 3

Cytotoxicity and in Vitro Antiprotozoal Activity of Dications **31**–**45**.



31 - 45

R = Am, *i*-PrAm, Im; R^1, R^2 = H, OMe, OH



Am ***i*-PrAm** **Im**

compd	R	R^1	R^2	Cytotoxicity	*T. brucei rhodesiense*[g]		*P. falciparum*[i]		*L. donovani*[k]	
				IC_{50} (μM)	IC_{50} (μM)	SI_T[h]	IC_{50} (μM)	SI_P[j]	IC_{50} (μM)	SI_L[l]
31	Am	H	H	86.0	0.006	14333	0.004	21500	17	5
32	*i*-PrAm	H	H	193	0.462	418	0.043	4488	58	3
33	Im	H	H	133	0.461	289	0.095	1400	>100	ND[m]
34	Am	OMe	H	>201	0.016	>12563	0.017	>11824	31	>6
35	*i*-PrAm	OMe	H	>173	0.447	>387	0.195	>887	>100	ND
36	Im	OMe	H	>170	0.162	>1049	0.022	>7727	>100	ND
37	Am	H	OMe	164	0.005	32800	0.007	23429	11	15
38	*i*-PrAm	H	OMe	>170	0.703	>242	0.034	>5000	32	>5
39	Im	H	OMe	>172	4.02	>43	0.064	>2688	>100	ND
40	Am	OH	H	>203	6.64	>31	1.23	>165	>100	ND
41	*i*-PrAm	OH	H	>170	15.1	>11	5.27	>32	>100	ND
42	Im	OH	H	>175	19.6	>9	0.885	>198	>100	ND
43	Am	H	OH	>210	0.008	>26250	0.007	>30000	8.0	>26
44	*i*-PrAm	H	OH	>175	0.077	>2273	0.049	>3571	>100	ND
45	Im	H	OH	>183	0.606	>302	0.016	>11438	>100	ND
PMD[a]				46.6	0.003	15533	0.058	803	1.8	25
MLSP[b]				7.78	0.004	1945				
CQ[c]				117			0.124	944		
ATMS[d]				450			0.006	75000		
PPT[e]				0.020						

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[a]PMD, Pentamidine.

[b]MLSP, melarsoprol.

[c]CQ, chloroquine.

[d]ATMS, artemisinin.

[e]PPT, podophyllotoxin.

[f]Cytotoxicity (L6 rat myoblast cells). Average of duplicate determinations. Maximum test concentration was 90 μg/mL. Based on the molecular weight of tested compounds, different > μM values were obtained from the conversion of 90 μg/mL to the μM scale.

[g]*Trypanosoma brucei rhodesiense* (STIB900). Average of duplicate determinations.

[h]Selectivity index for *T. brucei rhodesiense* (SI_T), expressed as the ratio [IC_{50} (L6)/IC_{50} (*T. brucei rhodesiense*)].

[i]*Plasmodium falciparum* (K1, resistant to chloroquine). Average of duplicate determinations.

[j]Selectivity index for *P. falciparum* (SI_P), expressed as the ratio [IC_{50} (L6)/IC_{50} (*P. falciparum*)].

[k]*Leishmania donovani* (MHOM/SD/62/1S-$CL2_D$) axenic amastigotes. Average of duplicate determinations.

[l]Selectivity index for *L. donovani* (SI_L), expressed as the ratio [IC_{50} (L6)/IC_{50} (*L. donovani*)].

[m]ND, not determined.

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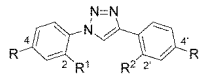
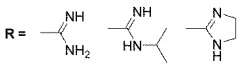
Table 4

Cytotoxicity and in Vitro Antiprotozoal Activity of Dications **46**–**60**.



46 - 60

R = Am, *i*-PrAm, Im; R^1, R^2 = H, OMe, OH



Am **i-PrAm** **Im**

compd	R	R^1	R^2	Cytotoxicity[f]	*T. brucei rhodesiense*[g]		*P. falciparum*[i]		*L. donovani*[k]	
				IC$_{50}$ (μM)	IC$_{50}$ (μM)	SI$_T$[h]	IC$_{50}$ (μM)	SI$_P$[j]	IC$_{50}$ (μM)	SI$_L$[l]
46	Am	H	H	8.10	0.004	2025	0.002	4050	9.1	<1
47	*i*-PrAm	H	H	>183	0.201	>910	0.005	>36600	25	>7
48	Im	H	H	101	0.053	1906	0.002	50500	>100	ND[m]
49	Am	OMe	H	>183	0.038	>4816	0.009	>20333	>40	ND
50	*i*-PrAm	OMe	H	>164	0.328	>500	0.019	>8632	>100	ND
51	Im	OMe	H	>176	0.675	>261	0.017	>10353	>100	ND
52	Am	H	OMe	59.4	0.031	1916	0.002	29700	13	5
53	*i*-PrAm	H	OMe	>166	0.201	>826	0.013	>12769	>100	ND
54	Im	H	OMe	142	0.116	1224	0.011	12909	>100	ND
55	Am	OH	H	>216	0.501	>431	0.170	>1271	>100	ND
56	*i*-PrAm	OH	H	>178	1.38	>129	0.800	>223	>100	ND
57	Im	OH	H	>198	0.998	>198	0.041	>4829	>100	ND
58	Am	H	OH	49.3	0.016	3081	0.002	24650	4.3	11
59	*i*-PrAm	H	OH	>180	0.037	>4865	0.007	>25714	>50	ND
60	Im	H	OH	>190	0.038	>5000	0.0006	>316667	24	>8
PMD[a]				46.6	0.003	15533	0.058	803	1.8	25
MLSP[b]				7.78	0.004	1945				
CQ[c]				117			0.124	944		
ATMS[d]				450			0.006	75000		
PPT[e]				0.020						

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[a]PMD, Pentamidine.

[b]MLSP, melarsoprol.

[c]CQ, chloroquine.

[d]ATMS, artemisinin.

[e]PPT, podophyllotoxin.

[f]Cytotoxicity (L6 rat myoblast cells). Average of duplicate determinations. Maximum test concentration was 90 μg/mL. Based on the molecular weight of tested compounds, different > μM values were obtained from the conversion of 90 μg/mL to the μM scale.

[g]*Trypanosoma brucei rhodesiense* (STIB900). Average of duplicate determinations.

[h]Selectivity index for *T. brucei rhodesiense* (SI_T), expressed as the ratio [IC_{50} (L6)/IC_{50} (*T. brucei rhodesiense*)].

[i]*Plasmodium falciparum* (K1, resistant to chloroquine). Average of duplicate determinations.

[j]Selectivity index for *P. falciparum* (SI_P), expressed as the ratio [IC_{50} (L6)/IC_{50} (*P. falciparum*)].

[k]*Leishmania donovani* (MHOM/SD/62/1S-$CL2_D$) axenic amastigotes. Average of duplicate determinations.

[l]Selectivity index for *L. donovani* (SI_L), expressed as the ratio [IC_{50} (L6)/IC_{50} (*L. donovani*)].

[m]ND, not determined.

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Table 5

Efficacy of Select Cationic Triazoles in the *T. brucei rhodesiense* STIB900 Mouse Model

compound	in vitro IC_{50} (nM)[a]	in vivo[b]		
		dose (mg/kg)[c]	cures[d]	MRD[f]
melarsoprol	4	4 × 8	4/4	–
		4 × 2	4/4	–
		4 × 1	2/4	20
pentamidine	3	4 × 20	2/4	26
		4 × 5	2/4	20
1	83	4 × 5	0/4	10
4	47	4 × 5	0/4	17
9	6	4 × 5	4/4	–
		4 × 1	0/4	12
		1 × 10	3/4	14
13	174	4 × 5	0/4	14.75
16	8	4 × 5	1/4	17.33
19	11	4 × 5	0/4	13
22	8	4 × 5	2/4	15.5
23	77	4 × 5	toxic[e]	–
24	21	4 × 5	0/4	26
25	175	4 × 5	0/4	14
28	6	4 × 5	4/4	–
29	116	4 ×5	0/4	28.75
30	43	4 × 5	0/4	10.75
31	6	4 × 5	1/4	18
34	16	4 × 5	1/4	23
37	5	4 × 5	4/4	–
		4 × 1	0/4	9.5
		1 × 10	0/4	23.5
43	8	4 × 5	2/4	39
44	77	4 × 5	1/4	18
46	4	4 × 5	4/4	–
		4 × 1	4/4	–
		1 × 10	3/4	27
		4 × 0.5	0/4	25.5
47	201	4 × 5	0/2[e]	8
48	53	4 × 5	0/4	8
49	38	4 × 5	0/4	34
52	31	4 × 5	3/4	60
58	16	4 × 5	4/4	–
59	37	4 × 5	4/4	–

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compound	in vitro IC_{50} (nM)[a]	in vivo[b]		
		dose (mg/kg)[c]	cures[d]	MRD[f]
60	38	4 × 5	0/4	10

[a] Average of duplicate determinations.

[b] STIB900 acute mouse model.

[c] Intraperitoneal administration.

[d] Number of mice that survive and are parasite free for 60 days.

[e] Mice died due to toxicity.

[f] Mean relapse day, untreated control mice have a high parasitamia load on day 7 and would expire between day 7 and 10 postinfection.