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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 15 (2005) 3592–3595

Synthesis of some diguanidino 1-methyl-2,5-diaryl-1*H*-pyrroles as antifungal agents

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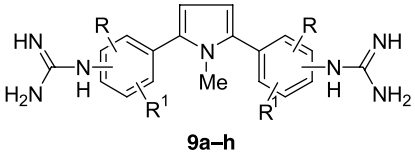
Received 28 March 2005; revised 10 May 2005; accepted 12 May 2005

Abstract—A series of novel 2,5-bis(guanidino-aryl)-1-methyl-1*H*-pyrroles **9a–h** has been synthesized starting from 1-methyl-1*H*-pyrrole. The antifungal activities of compounds were evaluated by in vitro agar diffusion and broth dilution assay against *Candida* spp. and *Aspergillus* spp. Compound **9c** from this series was found to be equipotent or more potent than fluconazole, whereas compound **9d** was comparable to fluconazole against most of the tested strains.

1. Introduction

The prevalence of systemic fungal infections, such as candidosis, cryptococcosis, and aspergillosis, has been escalating recently due to a raise in the number of immunocompromised hosts. For the treatment of these infections, dicationic compounds have been studied since the 1930s.[1] There are several reports in the literature about diamidine compounds, in which amidine groups have been used as cationic moieties, and their activities also against a number of pathogens including *Candida albicans*, *Aspergillus* sp., *Cryptococcus neoformans*, *Leishmania* sp., *Cryptosporidium parvum*, *Giardia lamblia*, *Plasmodium* sp., *Pneumocystis carinii*, *Toxoplasma gondii*, and *Trypanosoma* sp.[2–9] Diamidines exhibited a wide range of biological activity. One of the compounds of this group, pentamidine, has been reported to be used clinically. To improve further activities of this class of compounds, some guanidino compounds have been synthesized and evaluated for their antimicrobial activity.[10] Recently, there have been reports of antimicrobial (antifungal, antimycobacterial) activities of some diguanidino 2,5-diarylfuran compounds.[11] Unlike for amidines, which have been extensively studied, there are few reports[10,11] available on guanidines; therefore, this class of cationic compounds has yet to be explored further.



9a–h

Figure 1.

In our research work reported earlier,[12,13] we described the synthesis and antifungal activity of some tetrazole–triazole compounds. In view of the above and in continuation of our ongoing program to develop potent antifungal compounds, we have designed, synthesized, and evaluated antifungal activity of various diguanidino 1-methyl-2,5-diaryl-1*H*-pyrrole derivatives **9a–h** (Fig. 1), which we wish to report in this communication.

2. Chemistry

The six-step synthesis of derivatives **9a–h** is outlined in Scheme 1.

The syntheses of 2,5-bis(guanidino-aryl)-1-methyl-1*H*-pyrrole derivatives **9a–h** took place (Scheme 1) starting from 1-methyl-1*H*-pyrrole **2**,[14] which on reaction with tri-*n*-butyltin chloride in the presence of *n*-butyllithium and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine in refluxing

Keywords: Antifungal activity; 1-Methyl-1*H*-pyrrole; Diguanidino derivatives; Fluconazole.
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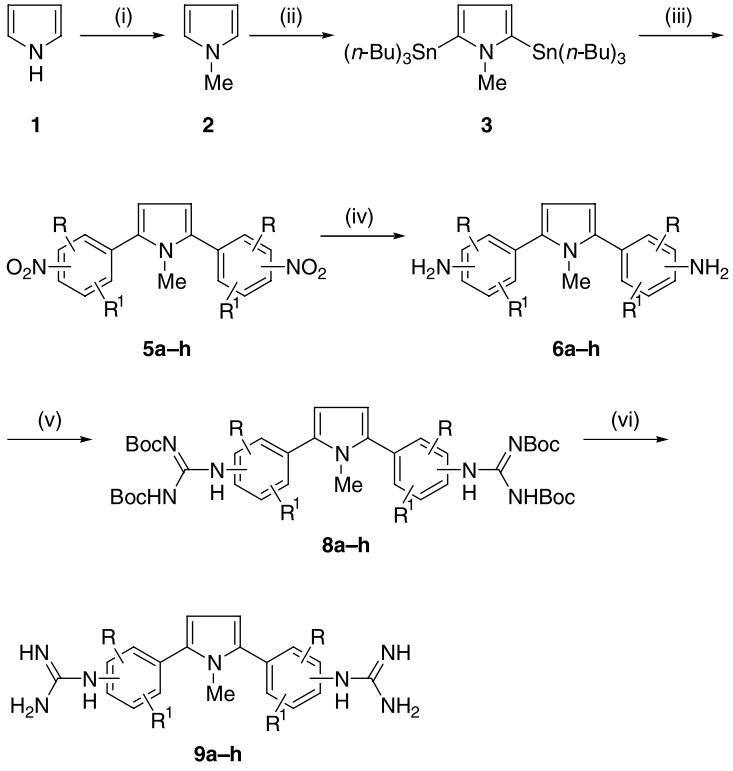
doi:10.1016/j.bmcl.2005.05.080

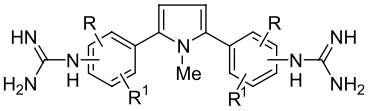


Scheme 1. Reagents and conditions: (i) Pulverized KOH, CH_3I, DMSO, 15–30 °C, 1–2 h; (ii) *n*-BuLi, tetramethylethylenediamine, $(n\text{-Bu})_3SnCl$, hexane, 1–2 h; (iii) bromonitroarene (**4**), $(PPh_3)_4Pd$, N_2 atmosphere, dioxane, 90–110 °C, 5–16 h; (iv) $SnCl_2{\cdot}H_2O$, EtOAc–H_2O (100:1), reflux, 1–2 h; (v) BocNH-C(=NBoc)SMe (**7**), $HgCl_2$, Et_3N, DMF, 25–30 °C, 24 h; (vi) HCl–EtOH, CH_2Cl_2, 0–25 °C, 70–84 h.

hexane gave 2,5-bis(tri-*n*-butylstannyl)-1-methyl-1*H*-pyrrole **3**. The compound **3** on Stille coupling[15] with substituted bromonitroarene **4** in the presence of tetrakis(triphenylphosphine)palladium(0) gave the corresponding nitro intermediates **5a–h**. The nitro derivatives **5a–h** were then reduced with tin(II) chloride dihydrate to obtain the amino compounds **6a–h**, which by reaction with Boc-protected *S*-methylthiourea **7** in the presence of mercury(II) chloride gave the Boc-protected diguanidino analogues **8a–h**. Deprotection of the Boc-protected guanidine analogues **8a–h** was carried out using ethanolic–HCl in dichloromethane at 0 °C to give the corresponding 2,5-bis(guanidino-aryl)-1-methyl-1*H*-pyrrole derivatives **9a–h** in good yield (Scheme 1, Table 1).[16]

All new compounds reported here were fully characterized on the basis of their ^1H NMR and MS spectroscopic and analytical data.[17]

Table 1. Range of MIC (μg/mL) values of compounds **9a–h** against clinical isolates of the fungal culture[a]



Compound	$NHC(=NH)NH_2$	R	R^1	Mp (°C)	C.a.I	C.a.II	C.k.I	C.k.II	C.p.	C.g.	C.t.	A.f.
9a	4-$NHC(=NH)NH_2$	H	H	285–287	>16	>16	>16	>16	>16	>16	>16	>16
9b	4-$NHC(=NH)NH_2$	2-OMe	H	226–228	16	16	16	16	16	16	16	16
9c[b]	4-$NHC(=NH)NH_2$	2-Me	H	282–284	**2**	**2**	**4**	**8**	**2**	**2**	**1**	**16**
9d	4-$NHC(=NH)NH_2$	2-Cl	H	246–248	8	8	16	16	16	16	4	>16
9e	4-$NHC(=NH)NH_2$	3-CF_3	H	250–252	16	16	16	16	16	16	4	16
9f	3-$NHC(=NH)NH_2$	4-F	H	326–328	16	16	16	16	16	16	16	16
9g	2-$NHC(=NH)NH_2$	4-Cl	H	210–212	>16	>16	>16	>16	>16	>16	>16	>16
9h	4-$NHC(=NH)NH_2$	2-F	4-F	160–162	>16	>16	>16	>16	>16	>16	>16	>16
Fluconazole					0.25	16	>8	32	8	>8	8	>64
Itraconazole					<0.03	0.25	0.25	<0.03	0.25	0.25	0.12	0.12

[a] C.a.I, *C. albicans* A261; C.a.II, *C. albicans* V-01-191 (resistant stain); C.k.I, *C. krusei* ATCC6528; C.k.II, *C. krusei* 1766-1; C.p., *C. parapsilosis* ATCC22019; C.g., *C. glabrata* ATCC90030; C.t., *C. tropicalis* ATCC750; and A.f., *Aspergillus fumigatus* LSI-11.

[b] Isolated as hydrochloride.

3. Biology

The antifungal activities of these new compounds **9a–h** were evaluated by in vitro agar diffusion and microbroth dilution assay and are summarized in Table 1. Most of the compounds showed activity against fungal cultures when tested at 500 μg/mL concentrations of compounds in the agar diffusion assay. These compounds were evaluated by microbroth dilution assay to determine the minimum inhibitory concentration (MIC) values in accordance with the guidelines in the NCCLS documents M27-A and M38-P.[18,19] In this assay, serial two-fold dilutions of the compounds were made to which a fixed volume ($1.5 \pm 1.0 \times 10^3$ cell/mL) of fungal cells (*Candida* species and *Aspergillus* species) was added and incubated at 25 °C for 48–72 h. The MIC values are expressed as the reciprocal of the highest dilution of the compounds showing 80% inhibition of the growth of the fungal culture. The results are expressed as means ± SD of the MIC values obtained in duplicate assay. The MIC values (in μg/mL) against *Candida* species and *Aspergillus* species in comparison with fluconazole and itraconazole are given in Table 1.

4-Guanidino compound **9c** having methyl substituents on the phenyl ring was the most active compound with an MIC value of 1–4 μg/mL for *Candida albicans*, *Candida krusei*, *Candida parapsilosis*, *Candida glabrata*, and *Candida tropicalis*. Compound **9d** having a chloro substituent on the phenyl ring also showed moderate activity against *Candida* species. Compounds **9a–b**, **e**, and **h** having different substitutions, such as H, OMe, CF_3, and F, on the phenyl ring did not show any activity against a test panel of fungal cultures. Complete loss of antifungal activity was observed in compounds **9f**, **g** having guanidine groups at 2- and 3-positions, respectively. None of the compounds were found to have any significant activity against *Aspergillus* species (Table 1).

4. Conclusions

In conclusion, a novel series of antifungal compounds that demonstrated significant activity against *Candida* species has been designed and synthesized. The antifungal activity of compound **9c** was better than that of fluconazole on *C. albicans*, *C. krusei*, *C. parapsilosis*, *C. glabrata*, and *C. tropicalis*. These findings clearly indicated that replacement of 2,5-diarylfurans with 2,5-diaryl-1-methyl-1*H*-pyrrole exhibits good antifungal activity compared to their furan analogs. This investigation indicates that there is potential to design and synthesize such types of compounds for the development of new antifungal compounds.

Acknowledgments

We wish to express our thanks to the Microbiology Department of New Chemical Entity Research, Lupin Research Park, Pune, for antifungal screening and to the Analytical Chemistry Department for ^{1}H NMR, mass spectroscopy, and elemental analyses of all the compounds synthesized.

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16. *Preparation of compound* ***3***. 1.6 M solution of *n*-BuLi (100 mL, 160 mmol) in hexane was added to a refluxing solution of **2**[14] (6.48 g, 80 mmol) and $(Me_2NCH_2)_2$ (18.56 g, 160 mmol) in hexane over a period of 1 h. The resultant reaction mixture was refluxed for 1 h and then treated with $(n\text{-Bu})_3SnCl$ (57.3 g, 176 mmol) dropwise. After an additional 2 h of refluxing, the cooled reaction

mixture was quenched with H_2O (100 mL) and extracted with hexanes (2 × 200 mL). The combined organic layer was dried (Na_2SO_4), filtered, and evaporated. Crude liquid was distilled under vacuum to yield **3** as a colorless oil (29 g, 55%, bp 205–210 °C at 1 mm Hg).

General procedure for Stille coupling. A mixture of substituted bromonitroarene (**4**, 15 mmol), $(PPh_3)_4Pd$ (0.52 mmol), and compound **3** (8 mmol) in anhydrous 1,4-dioxane (40 mL) was heated under a N_2 atmosphere at 90–100 °C for 5–16 h. The cooled reaction mixture was poured into H_2O (200 mL) and then extracted with EtOAc (2 × 100 mL), dried (Na_2SO_4), filtered, and evaporated. The crude product was purified by column chromatography over silica gel (100–200 mesh) using EtOAc–hexanes (1:4) as eluent to yield the corresponding products **5a–h**.

*General procedure for reduction of dinitro compounds **5a–h**.* A mixture of compound **5** (7 mmol), $SnCl_2{\cdot}2H_2O$ (70 mmol), and H_2O (1 mL) in EtOAc (100 mL) was refluxed for 1–2 h. The cooled reaction mixture was diluted with EtOAc (100 mL) and then basified with 20% NaOH (200 mL) and extracted with EtOAc (2 × 100 mL). The combined organic layer was dried (Na_2SO_4), filtered, and evaporated to yield a crude product, which was purified by column chromatography over silica gel (100–200 mesh) using EtOAc–hexanes (3:1) as eluent to give the corresponding diamino products **6a–h**.

*General procedure for the synthesis of compounds **8a–h**.* To a mixture of compound **6** (1 mmol), **7** (2.2 mmol), and Et_3N (6.6 mmol) in dry DMF (20 mL), $HgCl_2$ (2.2 mmol) was added and the resulting suspension was stirred at 25–30 °C for 24 h. The reaction mixture was diluted with EtOAc (50 mL) and filtered through a Celite pad. The filtrate was washed with 5% Na_2CO_3 solution (1 × 100 mL) and H_2O (1 × 100 mL). The combined organic layer was dried (Na_2SO_4), filtered, and evaporated to yield a crude product, which was purified by column chromatography over silica gel (100–200 mesh) using EtOAc–hexanes (1:9) to obtain pure products **8a–h**.

*General procedure for synthesis of compounds **9a–h**.* 6.43 M solution of HCl in EtOH (12 mL) was added to a solution of compound **8** (1.3 mmol) in CH_2Cl_2 (12 mL) at 0 °C and stirred at 25–30 °C for 70–84 h. Solvent was evaporated, crude mass was diluted with EtOAc, neutralized with 2 N NaOH (1 mL), and then evaporated to dryness to yield a crude product, which was purified by column chromatography over silica gel (100–200 mesh) using MeOH–$CHCl_3$ (1:4) as eluent to yield the corresponding products **9a–h**.

17. *Analytical data for compounds* **9a–h**. Melting points were determined in open capillaries on a Büchi B-545 melting point apparatus. ^{1}H NMR spectra were recorded on a Bruker Advance DRX 200 MHz instrument as solutions (DMSO-d_6) using TMS as internal reference, and chemical shift values are expressed in δ units. Mass spectra were run on an Applied Biosystems API 3000 instrument using a direct inlet system under positive ion electrospray ionization source. Elemental analyses were carried out on a Perkin-Elmer 2400 analyzer and the values were found to be within ± 0.4% of theoretical values. Compound **9a**: yellow solid (87%). ^{1}H NMR: δ 3.53 (s, 3H), 6.21 (s, 2H), 7.22 (d, J = 8.2 Hz, 4H), 7.47 (d, J = 8.2 Hz, 4H), 7.54 (br s, 6H), 10.11 (br s, 2H). MS: *m/z* 348 (M+1). Anal. Calcd for $C_{19}H_{21}N_7$ (347.42): C, 65.69; H, 6.09; N, 28.22%. Found: C, 65.47; H, 5.92; N, 28.54%. Compound **9b**: white solid (90%). ^{1}H NMR: δ 3.07 (s, 3H), 3.66 (s, 6H), 5.85 (s, 2H), 6.43 (d, J = 8.0 Hz, 2H), 6.51 (s, 2H), 6.99 (d, J = 8.0 Hz, 2H), 8.01 (br s, 8H). MS: *m/z* 408 (M+1). Anal. Calcd for $C_{21}H_{25}N_7O_2$ (407.47): C, 61.90; H, 6.18; N, 24.06%. Found: C, 62.05; H, 6.22; N, 23.90%. Compound **9c**: yellow solid (95%). ^{1}H NMR: δ 2.24 (s, 6H), 3.11 (s, 3H), 6.10 (s, 2H), 7.14 (d, J = 8.0 Hz, 2H), 7.22 (s, 2H), 7.36 (d, J = 8.0 Hz, 2H), 7.63 (br s, 6H), 10.21 (br s, 2H). MS: *m/z* 376 (M+1). Anal. Calcd for $C_{21}H_{25}N_7{\cdot}2HCl$ (448.39): C, 56.25; H, 6.07; N, 21.87%. Found: C, 56.20; H, 6.01; N, 21.88%. Compound **9d**: yellow solid (89%). ^{1}H NMR: δ 3.13 (s, 3H), 4.72 (br s, 2H), 6.06 (s, 2H), 7.04 (d, J = 8.0 Hz, 2H), 7.20 (s, 2H), 7.33 (d, J = 8.0 Hz, 2H), 7.91 (br s, 4H), 8.23 (s, 2H). MS: *m/z* 416 (M+1). Anal. Calcd for $C_{19}H_{19}Cl_2N_7$ (416.31): C, 54.82; H, 4.60; N, 23.55%. Found: C, 54.91; H, 4.69; N, 23.63%. Compound **9e**: yellow solid (93%). ^{1}H NMR: δ 3.59 (s, 3H), 6.43 (s, 2H), 7.49 (br s, 6H), 7.56 (d, J = 8.0 Hz, 2H), 7.78 (br s, 4H), 9.82 (br s, 2H). MS: *m/z* 484 (M+1). Anal. Calcd for $C_{21}H_{19}F_6N_7$ (483.41): C, 52.18; H, 3.96; N, 20.28%. Found: C, 53.96; H, 4.01; N, 20.25%. Compound **9f**: yellow solid (90%). ^{1}H NMR: δ 3.22 (s, 3H), 4.56 (br s, 6H), 5.91 (s, 2H), 7.37–7.42 (m, 6H), 9.12 (br s, 2H). MS: *m/z* 384 (M+1). Anal. Calcd for $C_{19}H_{19}F_2N_7$ (383.40): C, 59.52; H, 5.00; N, 25.57%. Found: C, 59.44; H, 5.13; N, 25.31%. Compound **9g**: white solid (97%). ^{1}H NMR: δ 3.10 (s, 3H), 4.87 (br s, 2H), 6.06 (s, 2H), 6.80 (d, J = 8.0 Hz, 2H), 7.20 (s, 2H), 7.23 (d,J = 8.0 Hz, 2H), 8.05 (br s, 4H), 8.40 (s, 2H). MS: *m/z* 416 (M+1). Anal. Calcd for $C_{19}H_{19}Cl_2N_7$ (416.31): C, 54.82; H, 4.60; N, 23.55%. Found: C, 54.69; H, 4.52; N, 23.50%. Compound **9h**: yellow solid (88%). ^{1}H NMR: δ 3.42 (s, 3H), 4.73 (br s, 8H), 6.29 (s, 2H), 7.08–7.33 (m, 4H). MS: *m/z* 420 (M+1). Anal. Calcd for $C_{19}H_{17}F_4N_7$ (419.38): C, 54.41; H, 4.09; N, 23.38%. Found: C, 54.46; H, 4.11; N, 23.20%.

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