SYNTHESIS OF 2,5-BIS-[4-(BENZHYDRAZIDO)-PHENYL]FURANS AND EVALUATION OF THE ANTIMALARIAL, TRYPANOCIDAL, AND CYTOTOXIC ACTIVITIES

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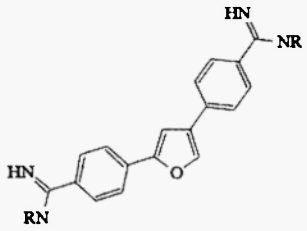
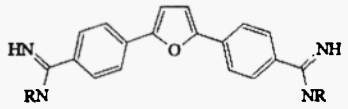
Abstract: 2,5-bis-[4-(benzhydrazido)-phenyl]furans were synthesized and evaluated for their inhibition of the *Trypanosoma vivax* cystein protease and their activity against cultured *P. falciparum* parasites, and their cytotoxicity against three cancer cell lines. Compounds **8**, **9** exhibited slight antimalarial activity, **8** was the most promising compound as cytotoxic against two cancer cell lines. Their activities against the *Trypanosoma vivax* cystein protease shown all to be inactive.

Introduction

Dicationic diaryl heterocyclic molecules are effective against a number of microbial infections including *Trypanosoma rhodesience* [1], *Pneumocystis carinii* [2], *Giardia lambia* [3], *Crystoporidium parvum* [4], *Plasmodium falciparum* [5], *Candida albicans* and *Crytococus neoformans* [6]. The mode of antimicrobial action for these dicationic molecules has been linked to their selective binding to the minor-groove of DNA at AT rich sites and their ability to selectively interfere with the normal functioning of one or more of several DNA dependents enzymes (topoisomerases, nucleases, etc), or possibly by direct inhibition of transcription [7-10]. A number of 2,5-diphenylfuran **1** and 2,4-diphenylfuran diamidines **2** have been found to be effective against *P. carinii, C. parvum* [2,11], and *T. rhodesience* [1]. Also, these diamidines have shown an *in vitro* antifungal activity against *C. albicans* and *C. neoformans* [6]. There are correlations between their binding to the AT-region in the dodecamer d(CGCGAATTCGCG), as shown by ΔTm measurements, the inhibition of DNA topoisomerase activity, and anti-PCP activity. Substitution of the amidino nitrogens with alkyl and cycloalkyl groups showed the largest increase in ΔTm. These alkyl derived furamidines have also been shown to have selective toxicity in several human ovarian tumour cell lines [12,13]. Cytotoxic potency is related both to the size of the alkyl group substituent, and to DNA binding affinity. This suggest that cytotoxic activity may be a consequence of binding to A/T-rich gene in DNA. In an effort to discover other biological activities such as antimalarial, antitumor and trypanocidal, we have focused our efforts on structural variations from the cationic center of these compounds. This report describe the synthesis and some in vitro antimalarial, and cytotoxic activities of dibenzhidrazido analogs in the diphenyl furan series. Their inhibition against the *Trypanosoma vivax* cystein protease were also evaluated



1



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Results and discussion

The key intermediate 2,5-bis(4-carboxyphenyl chloride)furan **4** required for making the final compounds **5-15** was synthesized from 2,5-bis-(cyanophenyl)furan **3**. 5-bis-[4-(benzylhydrazido)-phenyl]furans **5-15** were obtained via the diacid chloride 4 [2] by treatment with the corresponding phenylhydrazone. However, the bis nitrile **3** was prepared from 4-cyanobenzaldehyde by a more recently described two-step modified Stetter method involving the addition of divinyl sulfone to a solution of aldehyde in presence of a thiazolium catalyst [14] (scheme). No problem have been encountred in scaling up the procedure at a 5g size. All compounds have been characterized by IR, ^{1}H NMR and elemental analyses. The antimalarial activity was evaluated against a chloroquine resistant *P. falciparum* strain, the best antimalarial results were obtained with compounds **8** (IC_{50} of 67.3 μM) and **9** (IC_{50} of 87.3 μM) respectively. Compounds **5-7** and **10-15** have their activity at concentration higher than 100 μM (table 1). All compounds were also inactive against inhibition of cystein protease of *T. vivax*. Compounds **5-11** were tested against 3 cancer cell lines MCF7 (Breast), NCI-H460 (Lung), and SF-268 (CNS) at a concentration of 1.0 x 10^{-4} M, compound **8** was the most active against MCF7 (Breast), and SF-268 (CNS) cell lines (table 1). In summary, we found that compounds **8, 9** had a modest activity against *P. falciparum*, all compounds were inactive as trypanocidal; however, compounds **8** was the most promising from the 2,5-diphenylfuran series as cytotoxic against two cancer cell lines in this study.

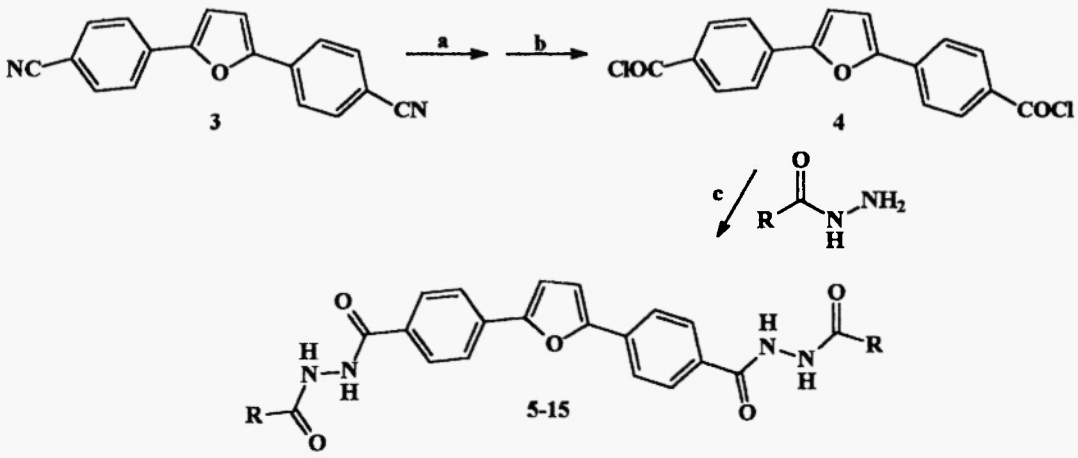
Experimental

Melting points were determined on a Thomas micro hot stage apparatus and are uncorrected. Infrared spectra were determined as KBr pellets on a Shimadzu model 470 spectrophotometer. The ^{1}H NMR spectra were recorded using a Jeol Eclipse 270 MHz spectrometer and are reported in ppm downfield from TMS (tetramethylsilane) as internal standard. Elemental analyses were performed by Atlantic Microlab, Norcross, GA, USA, results were within ± 0.4% of predicted values for all compounds. Chemical reagents were obtained from Aldrich Chemical Co. and Trans World Chemicals, USA. All solvents were distilled and dried with the usual desiccant. 2,5-bis-(4-cyanophenyl)furan **3** and 2,5-bis-(4-carboxyphenyl chloride)-furan **4** were synthesized as previously described [2, 14].

General procedure for the synthesis of 2,5-bis-[4-(benzhydrazido)-phenyl]furan **5-15**

To a solution of 300 mg (1.0 mmol) of 2,5-bis-(4-carboxyphenyl chloride)furan 4 in (10 ml) of chloroform was added triethylamine (0.1 ml), and then a solution of (2.0 mmol) of benzoylhydrazine respective in chloroform (1 ml). The mixture was stirred over night at room temperature, then concentrated, suspended in water, filtered, washed with water and then acetone and diethyl ether, recrytallized and dried in vacuo.

Scheme. Synthesis of 2,5-bis-[4-(benzhydrazido)-phenyl]furan derivatives **5-15**



a. MeOH, NaOH 30%, 24h, Δ. b. $SOCl_2$, benzene, DMF, Δ. c. $RCONHNH_2$, Et_3N, $CHCl_3$, rt.

2,5-bis-[4-(benzhydrazido)phenyl]furan **5**

Pale brown solid; yield 83%; m.p. 316-318 °C (DMSO/H_2O); IR (KBr): 3262, 1673, 1638 cm^{-1}; ^1H NMR (DMSO d_6): δ [ppm] 7.33 (2H, s, $H_{3,4}$), 7.53-7.61 (6H, m, $H_{3'',4'',5''}$), 7.92-7.94 (4H, m, $H_{2'',6''}$), 8.03 (8H, br s, $H_{2',3',5',6'}$), 10.55 (2H, s, NH), 10.60 (2H, s, NH). Anal. Calcd. For $C_{32}H_{24}N_4O_5$. $2H_2O$ C(66.22), H(4.85), N(9.64). Found C(65.97), H(5.02), N(9.46).

2,5-bis-[4-(2-methoxybenzhydrazido)phenyl]furan **6**

Yellow crystaline solid; yield 85%; m.p. 304-306 °C (DMSO/H_2O); IR (KBr): 3250, 1700, 1618 cm^{-1}; ^1H NMR (DMSO d_6): δ [ppm] 3.92 (6H, s, OMe), 7.09 (2H, dd, $H_{4''}$, J: 6.96, 7.68 Hz), 7.19 (2H, d, $H_{3''}$, J: 8.04 Hz), 7.34 (2H, s, $H_{3,4}$), 7.55 (2H, dd, $H_{5''}$, J: 7.52, 8.04 Hz), 7.78 (2H, d, $H_{6''}$, J: 7.72 Hz), 8.01 (8H, br s, $H_{2',3',5',6'}$), 10.08 (2H, s, NH), 10.73 (2H, s, NH). Anal. Calcd. For $C_{34}H_{28}N_4O_7$. $1/2H_2O$ C(66.54), H(4.73), N(9.13). Found C(66.25), H(4,59), N(8.96).

2,5-bis-[4-(3,4-dimethoxybenzhydrazido)phenyl]furan **7**

Pale brown powdery crystal; yield 76%; m.p. 300-302 °C (DMSO/H_2O); IR (KBr): 3264, 1673, 1641 cm^{-1}; ^1H NMR (DMSO d_6): δ [ppm] 3.84 (12H, s, OMe), 7.08 (2H, d, $H_{5''}$, J: 8.41 Hz), 7.34 (2H, s, $H_{3,4}$), 7.53-7.59 (4H, m, $H_{2'',6''}$), 8.03 (8H, s, $H_{2',3',5',6'}$), 10.39 (2H, s, NH), 10.53 (2H, s, NH). Anal. Calcd. For $C_{36}H_{32}N_4O_9$. 2 $1/2H_2O$ C(60.92), H(5.25), N(7.89). Found C(60.90), H(5.11), N(7.62).

2,5-bis-[4-(3,4,5-trimethoxybenzhydrazido)phenyl]furan **8**

Pale brown powdery crystal; yield 70%; m.p. 244-246 °C (DMSO/H_2O); IR (KBr): 3264, 1652, 1638 cm^{-1}; ^1H NMR (DMSO d_6): δ [ppm] 3.74 (6H, s, OMe), 3.86(12H, s, OMe), 7.28 (4H, s, $H_{2'',6''}$), 7.34 (2H, s, $H_{3,4}$), 8.03 (8H, s, $H_{2',3',5',6'}$), 10.49 (2H, s, NH), 10.59 (2H, s, NH). Anal. Calcd. For $C_{38}H_{36}N_4O_{11}$. $1/2H_2O$ C(56.01), H(5.07), N(6.87). Found C(56.13), H(4.72), N(6.84).

2,5-bis-[4-(4-chlorobenzhydrazido)phenyl]furan **9**

Cream powdery crystaline solid; yield 89%; m.p. 332-334 °C (DMSO/EtOH); IR (KBr): 3284, 1670, 1635 cm^{-1}; ^1H NMR (DMSO d_6): δ [ppm] 7.32 (2H, s, $H_{3,4}$), 7.61 (4H, d, $H_{3'',5''}$, J: 8.41 Hz), 7.94 (4H, d, $H_{2'',6''}$, J: 8.66 Hz), 8.01 (8H, s, $H_{2',3',5',6'}$), 10.62 (2H, s, NH), 10.64 (2H, s, NH). Anal. Calcd. For $C_{32}H_{22}N_4O_5Cl_2$. EtOH C(62.01), H(4.07), N(7.24). Found C(61.47), H(3.97), N(7.13).

2,5-bis-[4-(3-chlorobenzhydrazido)phenyl]furan **10**

Yelow crystaline solid; yield 72%; m.p. 296-298 °C (DMSO/EtOH); IR (KBr): 3248, 1676, 1638 cm^{-1}; ^1H NMR (DMSO d_6): δ [ppm] 7.33 (2H, s, $H_{3,4}$), 7.58 (2H, dd, $H_{5''}$, J: 7.91, 7.93 Hz), 7.68-8.00 (6H, m, $H_{2'',4'',6''}$), 8.02 (8H, s, $H_{2',3',5',6'}$), 10.67 (2H, s, NH), 10.69 (2H, s, NH). Anal. Calcd. For $C_{32}H_{22}N_4O_5Cl_2$. EtOH C(62.01), H(4.07), N(7.24). Found C(62.47), H(4.02), N(7.53).

2,5-bis-[4-(3-phenoxybenzhydrazido)phenyl]furan **11**

Whit solid; yield 94%; m.p. 278-280 °C (DMSO/H_2O); IR (KBr): 3282, 1671, 1639 cm^{-1}; ^1H NMR (DMSO d_6): δ [ppm] 7.07 (4H, d, $H_{2''',6'''}$, J: 8.16 Hz), 7.17-7.27 (4H, m, Ar), 7.30 (2H, s, $H_{3,4}$), 7.40-7.58 (8H, m, Ar), 7.69 (2H, d, $H_{6''}$, J: 7.67 Hz), 7.99 (8H, s, $H_{2',3',5',6'}$), 10.64 (4H, br s, NH). Anal. Calcd. For $C_{44}H_{32}N_4O_7$. 2I/2H_2O C(68.30), H(4.73), N(7.24). Found C(67.92), H(4.73), N(7.18).

2,5-bis-[4-(3-phenxylbenzhydrazido)phenyl]furan **12**

Cream fluffy solid; yield 74%; m.p. 362-364 °C (DMSO/H_2O); IR (KBr): 3250, 1680, 1665 cm^{-1}; ^1H NMR (DMSO d_6): δ [ppm] 7.34 (2H, s, $H_{3,4}$), 7.41-7.50 (10H, m, Ar), 7.77 (4H, d, $H_{2',6'}$, J: 7.15 Hz), 7.85 (4H, d, $H_{3',5'}$, J: 7.15 Hz), 8.03 (8H, s, $H_{2',3',5',6'}$), 10.61 (4H, br s, NH). Anal. Calcd. For $C_{44}H_{32}N_4O_5$. 1/2H_2O C(74.57), H(4.71), N(7.93). Found C(74.47), H(4.98), N(7.75).

2,5-bis-[4-(ethoxycarbonylhydrazido)phenyl]furan **13**

White crystalline solid; yield 90%; m.p. 250-252 °C (DMSO/H_2O); IR (KBr): 3250, 1730, 1650 cm^{-1}; ^1H NMR (DMSO d_6): δ [ppm] 1.22 (6H, t, CH_3), 4.08 (4H, q, CH_2), 7.30 (2H, s, $H_{3,4}$), 8.03 (8H, s, $H_{2',3',5',6'}$), 9.20 (2H, s, NH), 10.37 (2H, s, NH). Anal. Calcd. For $C_{24}H_{24}N_4O_7$. 2H_2O C(55.80), H(5.46), N(10.64). Found C(56.20), H(5.28), N(9.69).

2,5-bis-[4-(1-nafthyacylhydrazido)phenyl]furan **14**

Cream crystalline solid; yield 75%; m.p. 330-332 °C (DMSO/H_2O); IR (KBr): 3250, 1680, 1678 cm^{-1}; ^1H NMR (DMSO d_6): δ [ppm] 7.37 (2H, s, $H_{3,4}$), 7.59-7.67 (6H, m, $H_{3''',6''',7'''}$), 7.73 (2H, d, $H_{8'''}$, J: 6.68 Hz), 8.01-8.04 (4H, m, $H_{4'',5''}$), 8.10 (8H, s, $H_{2',3',5',6'}$), 8.48 (2H, d, $H_{2''}$, J: 7.67 Hz), 10.69 (4H, br s, NH). Anal. Calcd. For $C_{40}H_{28}N_4O_5$. 1I/2H_2O C(70.53), H(4.65), N(8.34). Found C(70.75), H(4.92), N(8.20).

2,5-bis-[4-(2-nafthyacylhydrazido)phenyl]furan **15**

Pale brown powdery solid; yield 71%; m.p. 320-324 °C (DMSO/H_2O); IR (KBr): 3242, 1671, 1683 cm^{-1}; ^1H NMR (DMSO d_6): δ [ppm] 7.35 (2H, s, $H_{3,4}$), 7.59-7.68 (4H, m, $H_{6'',7''}$), 7.97-8.03 (m, 8H, Ar), 8.06 (8H, s, $H_{2',3',5',6'}$), 8.58 (2H, s, $H_{1''}$), 10.73 (4H, br s, NH). Anal. Calcd. For $C_{40}H_{28}N_4O_5$. 2H_2O C(70.53), H(4.65), N(8.34). Found C(70.35), H(4.60), N(8.49).

Antimalarial Assays

The detailed protocol for in vitro antimalarial testing has been published previously [15-16].

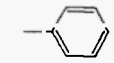
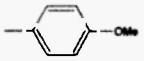
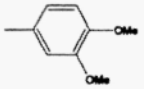
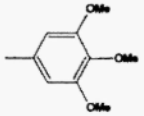
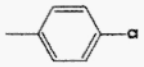
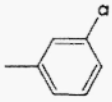
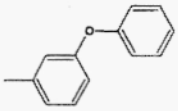
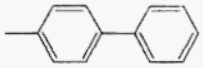
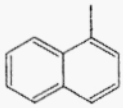
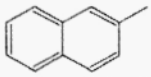
Cytotoxicity Assays

A reported methodology was employed to evaluate the cytotoxic potential of the compouns, using the cell lines MCF7 (Breast), NCI-H460 (Lung), and SF-268 (CNS) [17].

Assays of enzyme inhibition

Assays of the hydrolysis of the fluorogenic substrate benzyloxycarbonyl-Phe-Arg-7-amino-methyl-coumarin (Z-Phe-Arg-AMC) were performed. *T. vivax* extracts containing cystein proteinase were prepared as described previously [18].

Table 1. Antimalarial activity and cytotoxic potential of compounds **5-15**

		IC_{50} (μM) [a]	Grow Percentages $1x10^{-4}$ M		
No	**R**	**Parasite Development**	**MCF7 (Breast)**	**NCI-H460 (Lung)**	**SF-268 (CNS)**
5		> 100	146	125	199
6		> 100	84	61	78
7		> 100	58	109	253
8		67,3	107	19	23
9		87,2	113	107	133
10		> 100	116	103	135
11		> 100	108	92	98
12		> 100	100	80	101
13	—OEt	> 100	b	b	b
14		> 100	86	71	77
15		> 100	110	99	133

a IC_{50} ^{3}[H] hypoxanthine incorporated extrapolated from curves of percent Activity vs. concentration. b. Not tested

Acknowledgements

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