Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

Amidines bearing benzofuroxan or benzimidazole 1,3-dioxide core scaffolds as *Trypanosoma cruzi*-inhibitors: Structural basis for their interactions with cruzipain

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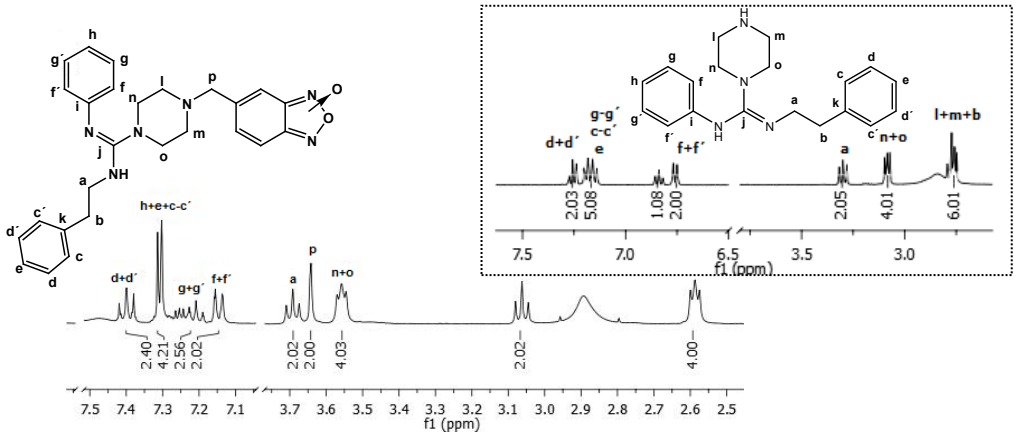
Study of the preferential tautomers for 4-10, and 19-22 in solution

From the ^{1}H NMR spectra of the amidine reactants and products some structural features regarding their structural disposition in solution, and at room temperature, could be figured out. In acetone-d_6 products **4-10** adopted the phenylazamethylidene tautomeric form whereas the reactants **19-22** preferred the R-ylazamethylidene form (as they are represented in Scheme 2) (Fig. S1a). Taking into account the deshielding of the protons in the lateral chain of the R group (Scheme 2) in products **4-10** respect to the same protons in reactants **19-22** and the chemical shift of the piperazine protons signals (Fig. S1b), it could be argued that amidine's aromatic substituents are differently arranged in reactants and products. In these last, substituents seem to establish intramolecular π-π-interactions, while in reactants the phenyl group extends upon the piperazine group. These hypothesis were confirmed by theoretical and experimental approaches. Theoretical conformational distribution studies in acetone were performed for amidines reactants and products at the DFT level (B3LYP/6.31G* with the continuum solvation model SM8). It was found that the most stable conformations, for each amidine-reactant, **19-22**, were those where the aryl systems did not establish π-π-interactions locating almost parallel to the piperazine ring, deshielding protons n and o (Fig. S1c). On the other hand, in each amidine-product, **4-10**, the aryl systems established π-π-interactions, as it is exemplified in Fig. S1c for compound **4**, in agreement with the shift of protons **a/b**. NOEdiff experiments confirmed these theoretical findings. Fig. S1d shows that for amidine **4** only aromatic protons (i.e. **d-g**) are spatially coupled in accordance with the disposition predicted theoretically.

a)



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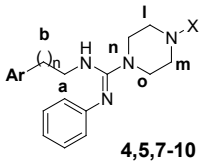
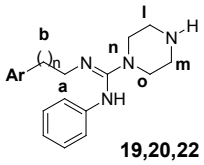


4,5,7-10

X= -CH_2-heterocycle
n= 0 or 1

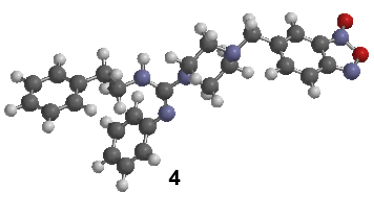


19,20,22

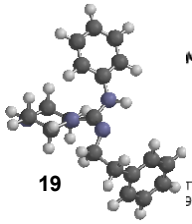
n= 0 or 1

Amidine product	δ (ppm) a/b	n,o	l,m	Amidine reactant	δ (ppm) a/b	n,o	l,m
4	3.69/3.06	3.55	2.59	**19**	3.30/2.77	3.08	2.77
8	3.57/2.44	3.50	2.62				
5	4.70	3.70	2.66	**20**	4.31	3.18	2.79
9	4.71	3.72	2.69				
7	4.76	3.63	2.64	**22**	4.23	3.17	2.80
10	4.81	3.46	2.56				

c)



4



19

d)

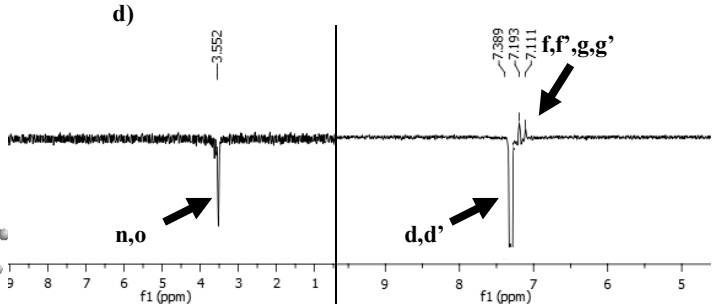


Figure S1. a) ^{1}H NMR spectra of amidine **4** in acetone-d_6 and at 303 K. Inset: ^{1}H NMR spectra of amidine reactant, **19**, in acetone-d_6 and at 303 K. Protons f, f', and h were used as indicators to determine the predominant tautomeric form in these conditions. **b)** Comparison of relevant proton chemical shifts, for Ha, Hb, Hn,o, and Hl,m, between amidine-products and -reactants. **c)** Examples of conformers for amidine-products and –reactants obtained by DFT-B3LYP/6.31G*-SM8 (acetone) calculus. **d)** Examples of NOEdiff experiments (for amidine **4**). Left: absence of nuclear overhauser effect when protons n,o were irradiated. Right: detection of nuclear overhauser effect on protons f-g when protons d were irradiated. Spectrum done in acetone-d_6 at 303 K acquired with mixing time of 250 ms.

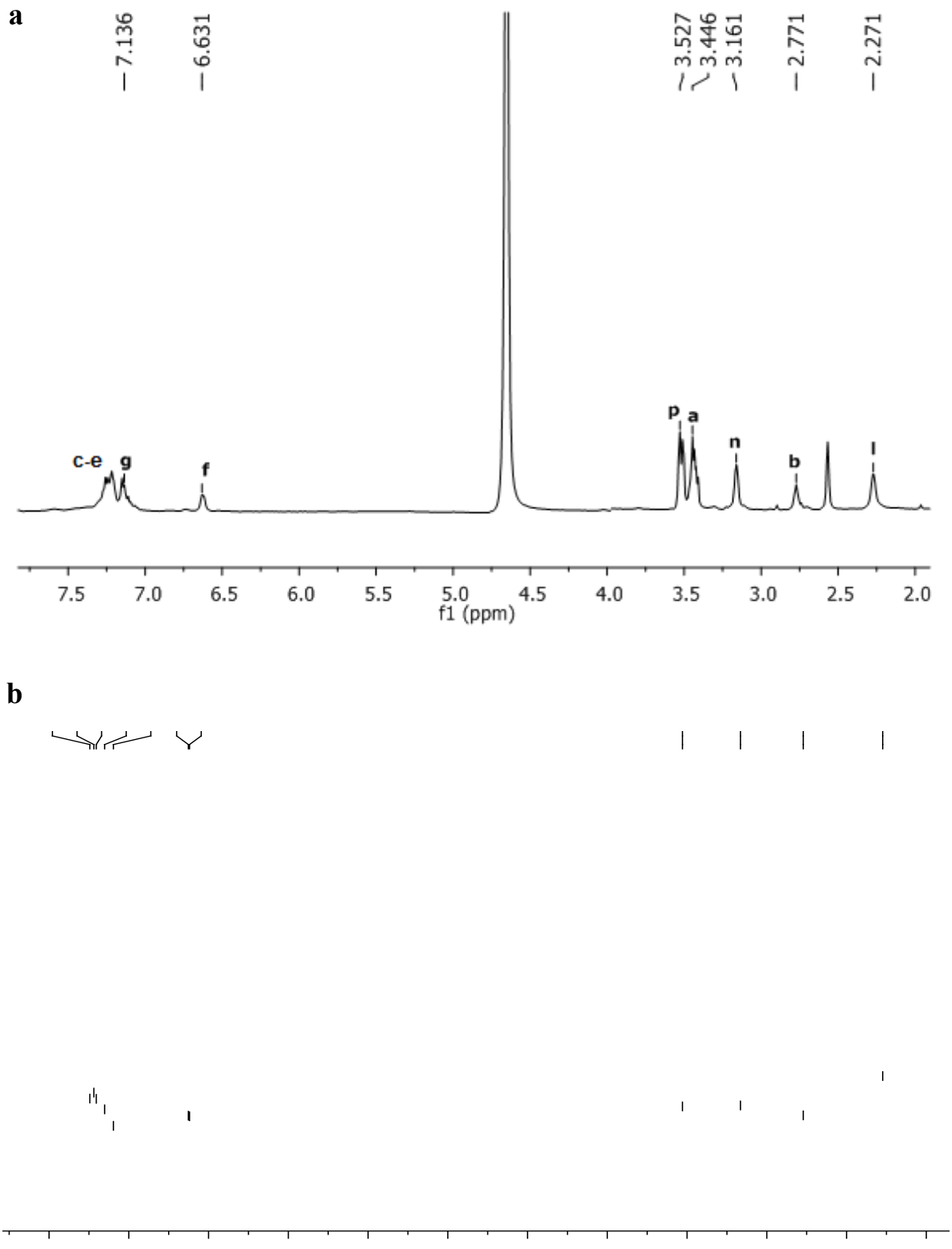


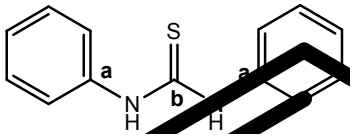
Figure S2. **a)** ^{1}H spectra of amidine **4** with CP **b)** The corresponding STD spectra.

Syntheis of thioureas 11-14

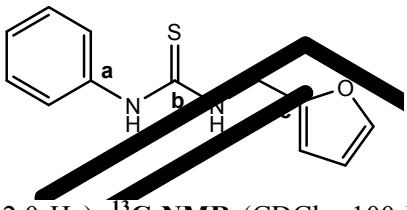
General procedure. To a solution of the corresponding amine (1 eq) in DMF (1 mL/mol amine) at 0 ºC was added phenylisothiocyanate (1 eq). The solution was stirred at room temperature during 12-24 h. Aqueous solution of HCl (10%) was added and extracted with EtOAc (3 × 50 mL). The organic phase was dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. The formed precipitate was filtered and washed with petroleum ether.

***N*-phenethyl-*N′*-phenylthiourea, 11**. White solid (61 %). **^{1}H-NMR** ($CDCl_3$, 400 MHz) δ (ppm): 2.94 (t, 2H, ^{3}J= 6.8 Hz), 3.91 (t, 2H, ^{3}J= 6.8 Hz), 7.02 (d, 2H, ^{3}J=7.2 Hz), 7.17 (d, 2H, ^{3}J=6.8 Hz), 7.24 (m, 2H), 7.32 (m, 4H). **^{13}C-NMR** ($CDCl_3$, 100 MHz) δ (ppm): 34.8 (CH_2), 46.4 (CH_2), 125.2 (Ar), 126.7 (Ar), 127.4 (Ar), 128.8 (Ar), 130.2 (Ar), 135.6 (Ca), 138.3 (Cc), 180.0 (Cb).

***N*-benzyl-*N′*-phenylthiourea, 12.** Pale brown solid (65 %). **^{1}H-NMR** (acetone-d_6, 400 MHz) δ (ppm): 4.90 (d, 2H, ^{3}J= 5.6 Hz), 7.23 (d, 2H, ^{3}J= 7.6 Hz), 7.34 (m, 6H), 7.43 (dd, 2H, $^3J_1$= 8.00 Hz, $^3J_2$= 7.6 Hz, ^{4}J= 2.0 Hz). **^{13}C-NMR** (acetone-d_6, 100 MHz) δ (ppm): 49.5 (CH_2), 125.4 (Ar), 127.5 (Ar), 127.7 (Ar), 127.8 (Ar), 128.9 (Ar), 130.2 (Ar), 135.8 (Ca), 137.2 (Cc), 181.3 (Cb).



***N*,*N′*-diphenylthiourea, 13.** Pale brown solid (63 %).**^{1}H-NMR** ($CDCl_3$, 400 MHz) δ (ppm): 7.32 (m, 2H), 7.43 (m, 8H), 8.25 (bs, 1H). **^{13}C-NMR** ($CDCl_3$, 100 MHz) δ (ppm): 125.4 (Ar), 127.3 (Ar), 129.7 (Ar), 136.9 (Ca), 161.6 (Cb).



***N*-phenyl-*N′*-furfurylthiourea, 14.** Pale brown solid (90 %). **^{1}H-NMR** ($CDCl_3$, 400 MHz) δ (ppm): 4.88 (d, 2H, ^{3}J= 3.6 Hz), 6.33 (m, 2H), 7.23 (dd, 2H, ^{3}J= 8.4 Hz, ^{4}J= 1.2 Hz), 7.33 (m, 2H), 7.44 (dt, 2H, $^3J_1$= 8.4 Hz, $^3J_2$= 6.4 Hz, $^4J_1$= 1.6 Hz, $^4J_2$= 2.0 Hz). **^{13}C-NMR** ($CDCl_3$, 100 MHz) δ (ppm): 42.1 (CH_2), 108.2 (Ar), 110.5 (Ar), 121.1 (Ar), 125.2 (Ar), 127.5 (Ar), 129.1 (Ar), 130.3 (Ar), 135.9 (Ca), 142.4 (Ar), 150.2 (Cc), 180.7 (Cb).

Synthesis of carbodiimides 15-18

General procedure. To a solution of the corresponding thiourea (1 eq) in CH_2Cl_2 (10 mL/mmol de tiourea) was added DIPEA (3 eq) and 2-chloro-*N*-methylpyridinium iodide (1.7 eq). The reaction mixture was stirred at room temperature until the complete disappearance of the thiourea was observed by TLC (SiO_2, petroleum ether:EtOAc 6:4). The reaction was evaporated *in vacuo* and the residue was filtered over SiO_2 and washed with petroleum ether:EtOAc 8:2. The filtrate was concentrated under reduced pressure to give the corresponding carbodiimide.

***N*-phenethyl-*N´*-phenylcarbodiimide, 15**. Yellow oil (95 %). **^{1}H-NMR** (acetone-d_6, 400 MHz) δ (ppm): 3.01 (t, 2H, ^{3}J= 6.8 Hz), 3.76 (t, 2H, ^{3}J= 6.8 Hz), 6.87 (dd, 2H, ^{3}J= 8.8 Hz, ^{4}J= 1.2 Hz), 8.00 (m, 1H), 7.31 (m, 7H). **^{13}C-NMR** (acetone-d_6, 100 MHz) δ (ppm): 37.2 (CH_2), 47.5 (CH_2), 123.0 (Ar), 124.7 (Ar), 126.4 (Ar), 128.5 (Ar), 129.0 (Ar), 129.3 (Ar), 135.8 (Cb), 138.8 (Cc), 141.0 (Ca).

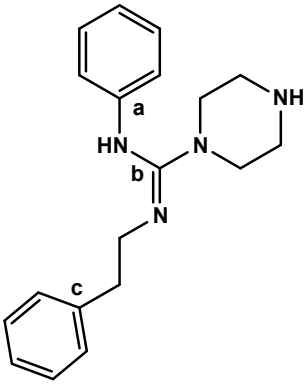
***N*-benzyl-*N´*-phenylcarbodiimide,16.** Brown oil (85 %). **^{1}H-NMR** (acetone-d_6, 400 MHz) δ (ppm): 4.67 (s, 2H), 7.03 (dd, 2H, ^{3}J= 8.2 Hz, $^4J_1$= 1.2 Hz, $^4J_2$= 0.8 Hz), 7.13 (t, 1H, $^3J_1$= 7.2 Hz, $^3J_2$= 7.6 Hz), 7.32 (m, 3H), 7.41 (t, 2H, $^3J_1$= 8.0 Hz, $^3J_2$= 6.8 Hz), 7.47 (d, 2H, ^{3}J= 6.8 Hz). **^{13}C-NMR** (acetone-d_6, 100 MHz) δ (ppm): 49.9 (CH_2), 123.4 (Ar), 124.8 (Ar), 127.6 (Ar), 127.7 (Ar), 128.7 (Ar), 129.4 (Ar), 137.1 (Cb), 138.4 (Cc), 140.4 (Ca).

***N,N´*-diphenylcarbodiimide, 17.** Brown oil (40 %). **^{1}H-NMR** (acetona-d_6, 400 MHz) δ (ppm): 7.24 (m, 6H), 7.41 (m, 4H).

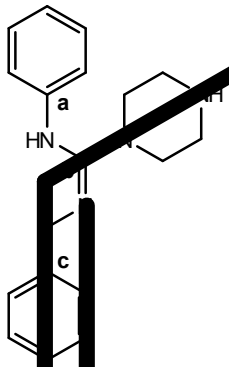
***N*-phenyl-*N´*-furfurylcarbodiimide, 18.** Yellow oil (64 %). **^{1}H-NMR** ($CDCl_3$, 400 MHz) δ (ppm): 4.53 (s, 2H), 6.34 (dd, 1H, ^{3}J= 3.2 Hz, ^{4}J= 0.8 Hz), 6.38 (dd, 1H, ^{3}J= 3.2, Hz, ^{3}J= 1.8Hz), 7.06 (m, 3H), 7.31 (dd, 2H, ^{3}J= 6.2 Hz, ^{4}J= 1.6 Hz), 7.42 (dd, 1H, ^{3}J= 1.8 Hz, ^{4}J= 0.8 Hz).

Synthesis of amidines reactants 19-22

General procedure. To a solution of the corresponding carbodiimide (1 eq) in toluene (10 mL/mmol carbodiimide) was added 1-(*tert*-butoxycarbonyl)piperazine (1 eq). The mixture was stirred at 50 °C until disappearance of the reactants was observed by TLC (SiO_2, CH_2Cl_2:MeOH 9:1). Toluene was evaporated *in vacuo* and the obtained product was used in the next step without purification. Deprotection process. To a solution of the above product (1 eq) in CH_2Cl_2 (10 mL/mmoL amidine) was added TFA (11 eq) and stirred at room temperature until disappearance of the reactive was observed by TLC (SiO_2, CH_2Cl_2:MeOH 9:1). Aqueous saturated solution of $NaHCO_3$ was added and the residue was extracted with CH_2Cl_2 (2 × 50 mL). The organic lawyer was dried over anhydrous Na_2SO_2 and was evaporated *in vacuo* affording the desired product.

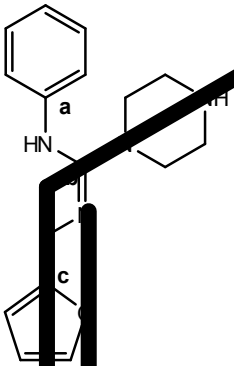


***N*-phenetyl-*N´*-phenylpiperazine-1-carboxamidine**, **19**. Yellow oil (96 %). **^{1}H-NMR** (acetone-d_6, 400 MHz) δ (ppm): 2.77 (m, 6H), 3.08 (t, 4H, ^{3}J= 4.8 Hz), 3.30 (t, 2H, $^3J_1$= 7.2 Hz, $^3J_2$= 7.6 Hz), 6.76 (dd, 2H, ^{3}J= 8.4 Hz, ^{4}J= 1.2 Hz), 6.84 (tt, 1H, $^3J_1$= 7.2 Hz, $^3J_1$= 7.6 Hz, ^{4}J= 1.2 Hz), 7.18 (m, 5H), 7.26 (m, 2H). **^{13}C-NMR** (acetone-d_6, 100 MHz) δ (ppm): 36.3 (CH_2), 45.7 (CH_2), 48.9 (CH_2), 120.3 (Ar), 121.8 (Ar), 126.02 (Ar), 128.3 (Ar), 128.7 (Ar), 139.6 (Cc), 150.6 (Ca), 155.4 (Cb).



***N*-benzyl-*N´*-phenylpiperazine-1-carboxamidine, 20**. Brown oil (100 %). **^{1}H-NMR** (acetone-d_6, 400 MHz) δ (ppm): 2.79 (t, 4H, ^{3}J= 4.8 Hz), 3.18 (t, 4H, ^{3}J= 4.8 Hz), 4.31 (s, 2H), 6.73 (dd, 2H, ^{3}J= 8.4 Hz, $^4J_1$= 1.2 Hz, $^4J_2$= 0.8 Hz), 6.83 (t, 1H, $^3J_1$= 8.4 Hz, $^3J_2$= 7.0 Hz), 7.16 (t, 2H, $^3J_1$= 8.0 Hz), 7.31 (dd, 5H, ^{3}J= 5.6 Hz, $^4J_1$= 2.8 Hz, $^4J_2$= 0.8 Hz). **^{13}C-NMR** (acetone-d_6, 100 MHz) δ (ppm): 45.6 (CH_2), 47.9 (CH_2), 49.0 (CH_2), 120.6 (Ar), 121.6 (Ar), 126.8 (Ar), 127.6 (Ar), 128.2 (Ar), 128.7 (Ar), 140.3 (Cc), 150.0 (Ca), 155.1 (Cb).

***N,N´*-diphenylpiperazine-1-carboxamidine, 21.** Pale brown solid (78 %). **^{1}H-NMR** (acetone-d_6, 400 MHz) δ (ppm): 2.80 (t, 4H, $^{3}J_1$= 5.2 Hz, $^{3}J_2$= 4.8 Hz), 3.30 (t, 4H, $^{3}J_1$= 5.2 Hz, $^{3}J_2$= 4.8 Hz), 6.86 (ddd, 2H, $^{3}J_1$= 6.8 Hz, $^{3}J_2$= 8.0 Hz, $^{4}J_1$= 1.2 Hz, $^{4}J_2$= 0.8 Hz), 6.93 (d, 3H, ^{3}J= 7.7 Hz), 7.17 (dd, 5H, ^{3}J= 7.8 Hz, ^{4}J= 1.2 Hz. **^{13}C-NMR** (acetone-d_6, 100 MHz) δ (ppm): 45.3 (CH_2), 45.8 (CH_2), 120.0 (Cc), 121.0 (Ar), 128.8 (Ar), 146.5 (Ca), 150.3 (Cb).



***N*-phenyl-*N´*-furfurylpiperazine-1-carboxamidine**, **22**. Brown oil (87 %). **^{1}H-NMR** (acetone-d_6, 400 MHz) δ (ppm): 2.80 (t, 4H, $^{3}J_1$= 5.2 Hz, $^{3}J_2$= 4.8 Hz), 3.17 (t, 4H, $^{3}J_1$= 5.2 Hz, $^{3}J_2$= 4.8 Hz), 4.23 (s, 2H), 6.22 (dd, 1H, ^{3}J= 3.2 Hz, ^{4}J= 0.8 Hz), 6.36 (dd, 1H, $^{3}J_1$= 3.2 Hz, $^{3}J_2$= 1.8 Hz,), 6.72 (d, 2H, ^{3}J= 7.2 Hz), 6.85 (t, 1H, ^{3}J= 7.2 Hz), 7.17 (t, 2H, $^{3}J_1$= 7.6 Hz, $^{3}J_2$= 8.0 Hz), 7.47 (dd, 1H, ^{3}J= 1.8 Hz, ^{4}J= 0.8 Hz). **^{13}C-NMR** (acetone-d_6, 100 MHz) δ (ppm): 41.3 (CH_2), 45.6 (CH_2), 48.9 (CH_2), 106.7 (Ar), 110.3 (Ar), 120.6 (Ar), 121.4 (Ar), 128.8 (Ar), 141.8 (Ar), 149.8 (Ca), 153.5 (Cc), 154.9 (Cb).