Design, Synthesis and Activity Against *Trypanosoma cruzi* of Azaheterocyclic Analogs of Megazol

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Abstract: This study describes the design, synthesis and trypanocidal evaluation of new azaheterocyclic derivatives (**4-8**). These compounds were designed as megazol (**1**) analogs based on bioisosterism tools and were synthesized to investigate the possible pharmacophoric contribution of the 1,2,4-triazole nucleus, the position of the heterocyclic nucleus and presence of the nitro group, to the activity against the bloodstream trypomastigote forms of *Trypanosoma cruzi*. The most potent compound was **6**, a nitro derivative obtained by substitution of a thiadiazole by a triazole ring and by moving the nitro group from C-5 position, as in **1**, to the C-4 position. Finally, we have used semi-empirical theoretical calculations to discuss the correlation of some stereo electronic properties with biological activity in an attempt to understand the possible mechanism of action of the designed series of compounds.

Key Words: Azaheterocyclic derivatives, megazol, *Trypanosoma cruzi*, Chagas disease, chemotherapy.

INTRODUCTION

Megazol (**1**), a 5-nitroimidazole initially produced by American Cyanamid Co. [1], has proven to have high efficacy both *in vitro* and *in vivo* against *Trypanosoma cruzi* [2,3] and *Trypanosoma brucei* [4,5], the causative agents of Chagas disease and African trypanosomiasis, respectively. Taking into account the limited efficacy and severe side effects of nifurtimox and benznidazole, the currently available drugs for Chagas disease, **1** represents a promising therapeutic alternative [6]. Studies on the mechanism of action of **1** against *T. cruzi* indicate interference in oxygen metabolism [7], inhibition of NADH fumarate reductase activity [8] and scavenging of intracellular thiols [9], specially trypanothione, specific substrate for trypanothione reductase, involved in detoxification processes. Inspection of the structure of **1** shows a recognized chemical group, that is –N=C-NH_2, responsible for purine transport [8]. Despite its remarkable *in vivo* activity, including against *T. cruzi* strains resistant to nifurtimox or benznidazole [3], **1** was shown to possess *in vitro* mutagenic and genotoxic effects [10-12]. In this context, the suitability of megazol as a chemotherapeutic agent for Chagas disease requires further extensive investigation. Trying to circumvent this undesired profile, different series of analogs of **1** were prepared and assayed against *T. cruzi* [13,14]. In this context, we have previously synthesized and analyzed the trypanocidal profile of 5-[N-(3-(5-trifluoromethyl)-1H-1,2,4-triazolyl)]amino-1-methyl-4-nitroimidazole (**2a**), 5-[N-(3-(5-trifluoromethyl)-1,3,4-thiadiazolyl)] amino-1-methyl-4-nitroimidazole (**2b**) and 5-N-(1-pyrazolyl)-1-methyl-4-nitroimidazole (**3**) [15,16] (Fig. **1**).

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CHEMISTRY

We designed a new class of azaheterocyclic derivatives related to **1** (**4-8**). This series was structurally planned, aiming to introduce further modifications **2a** and **3**, previously reported as active against *T. cruzi* [15,16] (path A, Fig. (**1**)). Due to the biological results obtained [14-16], we have assumed that the triazole ring introduced in **4** and **6**, which represents an isosteric replacement, could imitate the thiadiazole system of **1** and improve the trypanocidal activity as shown in **2a** and **2b** [16]. The structural modifications of this series involved the introduction of a triazole ring (**4** and **6**), and a change in the position of nitro and triazole or thiadiazole groups in **5-8**, since it has been previously reported that the incorporation of substitutents at the α position relative to the nitro group of the imidazole ring of **1** generally resulted in a large reduction of mutagenicity, without interfering with the pharmacological activity [17].

The azaheterocyclic 5-(1-methyl-5-nitro-1*H*-2-imidazolyl)-1H-1,2,4-triazol-3-amine (4) was prepared by the condenzation of 2-cyano-1-methyl-4-nitroimidazole (9) [14,18] with aminoguanidine (Scheme **1**). The compounds 5-(1-methyl-4-X-1H-5-imidazolyl)-1,3,4-thiadiazol-2-amine, where X=NO_2 (5) or X=NH_2 (7) and 5-(1-methyl-4-X-1H-5-imidazolyl)-1H-1,2,4-triazol-3-amine, where X=NO_2 (6) or X=NH_2 (8), were prepared by condenzation between 5-cyano-1-methyl-4-nitroimidazole (10) and thiosemicarbazide or aminoguanidine (see Scheme **1**). The intermediate 10 was prepared in two steps: nitration of commercial 5-chloro-1-methylimidazole with sodium nitrate in sulphuric acid, giving 5-chloro-4-nitro-1-methylimidazole, which was then treated with potassium cyanide in aq. DMSO [19] (Scheme **1**). The compounds (4) to (8) were purified by recrystallization in ethanol and were fully characterized by ^{1}HNMR, ^{13}CNMR, IR and MS spectroscopies.

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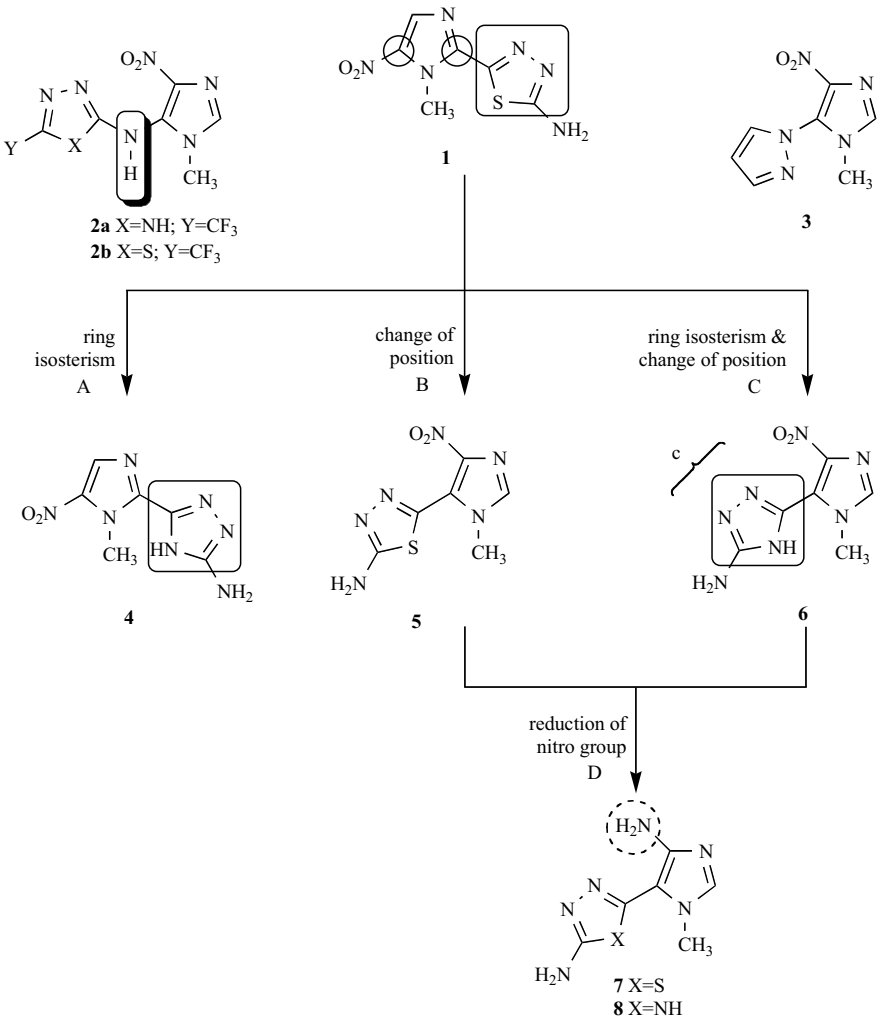


Fig. (1). Design concept of megazol analogs.

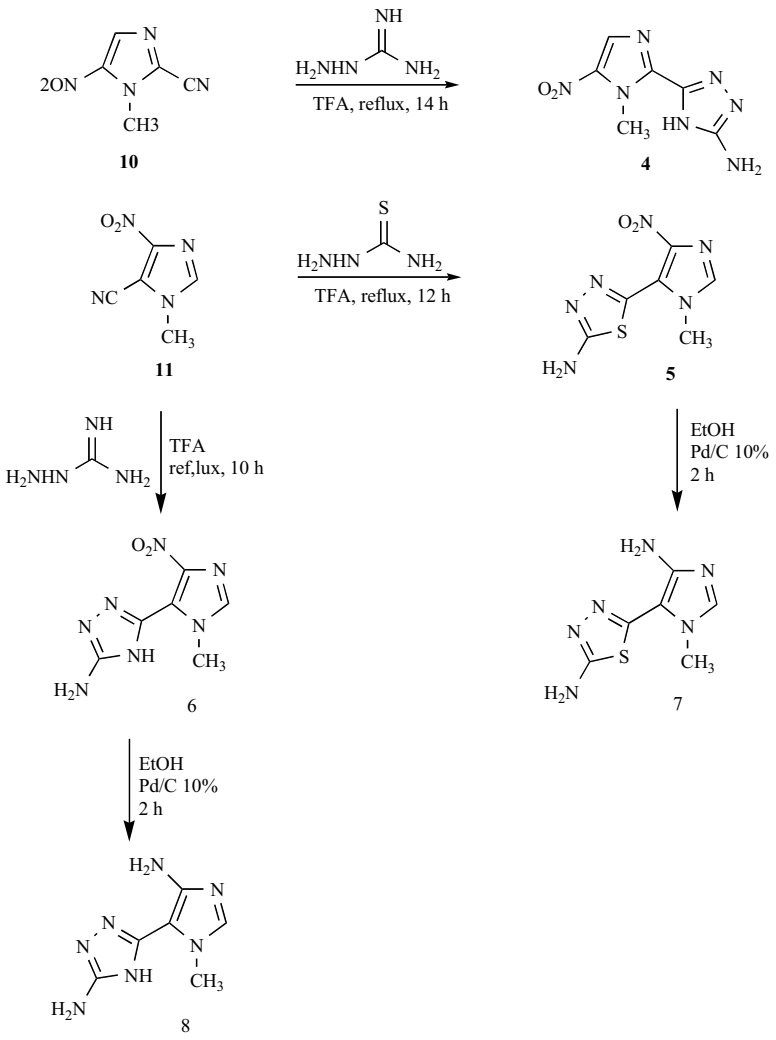
ANTI-TRYPANOSOMAL ACTIVITY

Bloodstream trypomastigotes of the Y_6 strain [20] were resuspended to a concentration of 10 x 10^6 cells/mL in Dulbecco's modified Eagle medium plus 10% fetal calf serum. This suspension (100 µL) was added to the same volume of each compound, previously prepared at twice the desired final concentrations. Incubation was performed in 96-well microplates at 4°C for 1 day. Untreated and megazol-treated parasites were used as controls. The final concentration of DMSO did not exceed 0.2%, which caused no damage to the parasites [21]. Cell counts were performed in Neubauer chamber and the activity of the compounds was expressed as IC_{50}/1 day corresponding to the concentration that led to 50% lysis of the parasites.

COMPUTATIONAL STUDIES

Theoretical calculations were performed in order to investigate the stereoelectronic properties of megazol (1) and to compare them with the properties of the azaheterocyclic derivatives (4) to (8), trying to establish some SAR, since the number of molecules synthesized would not be enough for QSAR studies. Thus, these studies aimed at identifying physical-chemical properties that may be involved in the mechanism of action of these compounds as a tool for further structural modification in this series. The properties of interest in this study were theoretical log P (clog *P*) [22a], polar superficial area (PSA) [22b], HOMO and LUMO energies and single-electron reduction potentials [22c] that were correlated to pIC_{50} (-LOGIC_{50}) (Table **1**). Log P values may give some insight on the degree of hydrophobicity of these molecules and PSA indicates the hydrogen bonding potential of the molecules. Additionally, HOMO and LUMO energies and single-electron reduction potentials may shed some light on the electronic features of the designed molecules that are important for bioactivity. All calculations were performed in Sybyl v6.8 [22d] and graphics were obtained with Hyperchem, demo version 7 [22e].

All computational procedures were performed on an Origin workstation under the operational system IRIX64, release 6.5 (CPU R10000). All graphics were obtained with Hyperchem, demo version 7. Molecules were built using the "Sketch Molecule" module, in Sybyl v6.8 and were energy



Scheme (1). Synthetic route for the preparation of the azaheterocyclic derivatives 4 to 8. TFA: trifluoroacetic acid; r.t. = room temperature.

minimized using Gasteiger-Hückel charges and Tripos molecular mechanics force field. Next, conformational analysis was performed using the "Systematic Search" module, in Sybyl v6.8. All flexible bonds were systematically varied with increments of 30°. HOMO and LUMO energies were obtained after full optimization of the sets of conformations found in the previous step, upto a GNORM of 0.01, using the AM1 Hamiltonian module. Single-electron reduction potentials were calculated from these structures, according to the following scheme: $\Delta G(L)^{\cdot-} = G(L)^{\cdot-} - G(L)$, where $\Delta G(L)^{\cdot-}$= the single-electron reduction potential; $G(L)^{\cdot-}$= energy of the radical anion species and **G(L)**= total energy of the neutral molecule. The calculations were obtained in the gas phase at 298°K. The keywords used were: FORCE THERMO= 298,298 ROT=1 DOUBLET. LOGP values were calculated with the CLOGP program and PSA values were calculated using MOLPROP, in Sybyl/QSAR module.

RESULTS AND DISCUSSION

The data related to the trypanocidal activity and stereo electronic parameters are displayed in Table **1**. Compound (6), a position analog of 1 containing a triazole group, was the most potent of the series, with an IC_{50}/1 day of 38.6 ± 8.7 μM. Comparing 8 with 6, it can be observed that the substitution of a nitro group by an amino group in the molecule led to a drastic decrease of the trypanocidal activity, suggesting the involvement of reactive oxygen species on the effect against *T. cruzi*. Unexpectedly, compound (4), that was designed with a triazole ring in substitution to the thiadiazole ring of 1, keeping the nitro group at the C-2 position of the imidazole ring, was 53- and 13-fold less potent than 1 and 6 respectively. These results suggest that there is a differential conformational *ortho* effect of the methyl and nitro groups, concerning the alignment of the two heterocyclic rings present in 6 as compared to 4, which may be one of the causes of the decrease in the biological activity. Compounds (5), (7) and (8) were also less active than 1 in the same experimental conditions.

HOMO energies of 1, and the designed compounds (4-8) were very close, in general. However, 1 has the most favorable value (-9.35 eV) and it is observed that compounds 5, 7

Table 1. Experimental IC_{50} Values for Megazol (1) and the New Azaheterocyclic Derivatives (4-8) Against Trypomastigotes of *Trypanosoma cruzi,* Calculated Stereo Electronic Parameters and pIC_{50}

Compound	IC_{50}/1d[a] (μM)	clog *P*[b]	PSA[c] (Å^2)	E_{HOMO}[d] (eV)	E_{LUMO}[d] (eV)	ΔG(L)$^{-}$[e] (kcal/mol)	pIC_{50}[f]
1	9.9 ± 0.8	-0.30	261.80	-9.35	-1.49	-62.28	5.00
4	522.4 ± 56.2	-0.49	251.86	-9.20	-1.32	-51.29	3.28
5	>4000	-0.54	243.84	-8.95	-1.38	-52.57	2.40
6	38.6 ± 8.7	-0.73	242.79	-9.09	-1.09	-48.16	4.41
7	>4000	-0.63	214.82	-8.23	-0.87	-40.64	2.40
8	849.3 ± 101.6	-0.82	219.97	-8.39	-0.16	-27.65	3.07

[a]Mean ± S.D. deviation of at least 3 experiments; [b]Log of the octanol/water partition coefficient; [c]Polar Surface Area, calculated as the surface area of all O/N/S atoms and hydrogens covalently bonded to these atoms; [d]Molecular orbital energies; [e]Gibbs free energy of single-electron reduction of ligand; [f]pIC_{50}= -LOGIC$_{50}$. The activity of **5** and **7** has been fixed at 4000 μM for correlation purposes.

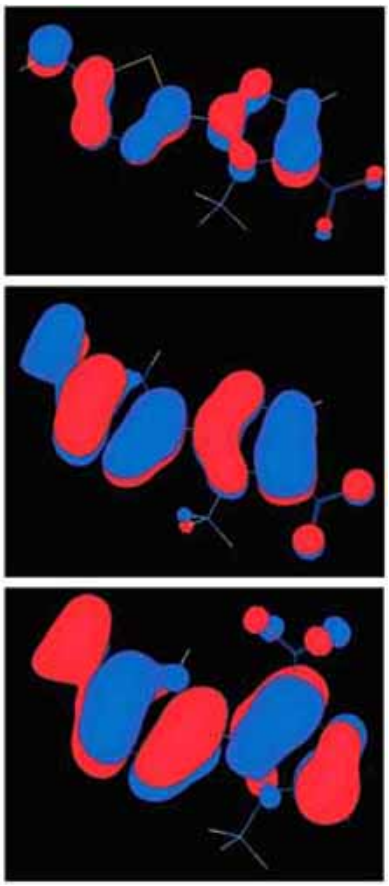
and 8, that lie among the less active compounds, did not present HOMO values as favorable (low) as compounds 1, 4 and 6 (Table **1**). Additionally, analysis of LUMO energy values shows that all compounds but 3, 7 and 8 had similar energy values. Interestingly, when we compare the PSA values, that correspond to the polar surface area of the molecules and is related to hydrogen bonding ability, 1, the most active compound, has the highest value, while 7 and 8 have the lowest values (Table **1**). Finally, analyzing the clog *P* values we have found no correlation with biological activity. In an attempt to establish some correlation of these parameters with biological activity, we built a simple correlation matrix of these stereo electronic properties (data not labeled), and found that there is a reasonable correlation between PSA and pIC_{50} (R= 0.66). The data suggest that the polar surface area of the designed compounds described in this work is important for interaction with their biological target, as suggested before, specifically by means of hydrogen bonding. Also, there is an inverse correlation of HOMO (R= -0.68) and single-electron reduction potential with biological activity (pIC_{50}) (R= -0.51). These two properties are highly inter-correlated (R= 0.85) as are practically all the other properties. These data suggest that the designed compounds described in this work may be taking part in redox reactions with important proteins of the parasite's life cycle. Additionally, since there is an inverse correlation, lower values of HOMO energies or single-electron reduction potentials contribute to a higher activity. This could partially explain the bad performance of 5, 7 and 8, although it must be emphasized that this property alone does not explain the whole activity observed in this study and this result is expected, since the biological assays have been performed in cells, demanding a series of adequate molecular properties for the observation of activity, such as a good membrane permeation. Finally, when we compare the HOMO density plots of 1, 4 and 6 (Fig. **2**), it is clear that there is a localized electronic density in the $–NH_2$ group of 1. Nevertheless, there is a greater distribution of electronic density between the two heterocycles in 4 and 6 as compared to 1. On the other hand, 6, in comparison to 4, has a greater electronic distribution localized on the substituent $–NH_2$ and on the –NH group of the triazole ring, facilitated by the slight out-of-the plane ring conformation and electrostatic attraction between the hydrogen atom of the –NH group of the triazole ring and one of the oxygen atoms of $–NO_2$ attached to the imidazole ring (Fig. **2**), which pulls electrons out of the triazole ring. One must remember that the HOMO energy characterizes the ability of electron-giving and may be used to represent the free radical scavenging ability of molecules. Also, it is a π-like orbital that can be delocalized in the molecule, and the same is true for LUMO. Therefore, the differential electronic delocalization between 1, 4 and 6 may partially account for the difference in the biological activity of these compounds, although other parameters may not be excluded.

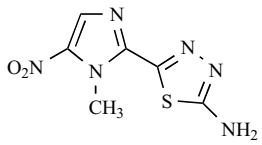
CONCLUSIONS

In this work, we have synthesized new azaheterocyclic derivatives, megazol analogs. Although the observed antitrypanosomal activity for 6 was not higher than 1, it was possible to use theoretical calculations to discuss the correlation of some stereo electronic properties, like HOMO energies and PSA, with pIC_{50}, as a means to understand the possible mechanism of action of the designed series of molecules. Furthermore, new compounds will be synthesized, in order to understand the molecular parameters essential for the trypanocidal activity and decide which properties must be improved in order to optimize this class of compounds.

EXPERIMENTAL SECTION

All reactions were monitored by thin-layer chromatography over pre-coated Silica gel 60 F254 plates (Merck, Darmstadt, Germany) and visualized by UV irradiation. The CG-MS spectra were obtained on a Hewlett Packard 5890 gas chromatograph connected to a Hewlett Packard 5960 MS (Hewlett Packard Co., Palo Alto, CA, USA).The fragments were described as the relation between atomic mass units and the charge (m/z) and the relative abundance in percentage of the base peak intensity. ^1H and ^{13}C NMR were recorded in a Bruker 200 MHz spectrometer (Bruker-Franzen Analytic, Bremen, Germany), using DMSO-d6 as solvent, with TMS as internal standard. Chemical shifts (δ) are given in ppm. The abbreviation sb refers to singlet broad signal. Elemental analysis was performed in a Perkin-Elmer Model EA 2400





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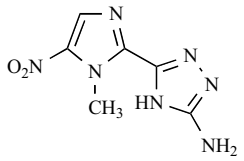

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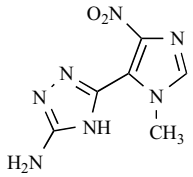

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Fig. (2). HOMO density distributions of megazol (**1**) and compounds **4** and **6**.

CHN (Perkin-Elmer Inc., Wellesley, MA, USA), and the results agreed within ± 0.4% of the calculated compositions.

5-(1-Methyl-5-nitro-1H-2-imidazolyl)-1H-1,2,4-triazol-3-amine (4)

Aminoguanidine hydrochloride (11 mmol) plus compound (9) (11 mmol) were heated in trifluoroacetic acid (5 mL) at 60° C for 15 h. The reaction medium was poured into ice-water (10 mL) and neutralized with saturated $NaHCO_3$ aqueous solution. The precipitate obtained was filtered, washed with water (2X5 mL), and dried under vacuum. Compound (4) was obtained as white solid (65% yield). MS (70 eV, *m/z*) 209 (M+, 100%), 179 (M+ -30, 10%), 167 (M+ -42, 39%), 139 (M+ -70, 90%); ^{1}H-NMR δ 4.20 (s, 3H, N-CH_3), 8.05 (sb, 2H, change with D_2O, NH_2), 8.17 (s, 1H), 8.20 (sb, 1H, change with D_2O, NH); ^{13}C-NMR δ 34.54 (N-CH_3), 130.80 (C4), 140.56 (C5), 141.32 (C2), 148.20 (C3*), 169.73 (C5*); Anal. calcd. for $C_6H_7N_7O_2$: C 34.45, H 3.37, N 46.88. Found: C 34.49, H 3.35, N 46.68.

5-(1-Methyl-4-nitro-1H-5-imidazolyl)-1,3,4-thiadiazol-2-amine (5)

Thiosemicarbazide (11 mmol) plus compound (10) (11 mmol) were heated in trifluoroacetic acid (5 mL) at 60° C for 15 h. The reaction medium was poured into ice-water (10 mL) and neutralized with saturated $NaHCO_3$ aqueous solution. The precipitate obtained was filtered, washed with water (2X5 mL), and dried under vacuum. Compound (5) was obtained as yellow solid (58% yield), according to the procedure described above. MS (70 eV, *m/z*): 226 (M+, 100%), 211 (M+ -15, 28%), 196 (M+ -30, 49%), 180 (M+ -46, 35%), 101 (M+ -125, 65%); ^{1}H-NMR δ 4.00 (s, 3H, N-CH_3), 7.71 (sb, 2H, change with D_2O, NH_2), 7.93 (s, 1H); ^{13}C-NMR δ 34.30 (N-CH_3), 127.15 (C4), 138.27 (C5), 142.70 (C2) 148.21 (C2*), 169.50 (C5*); Anal. calcd. for $C_6H_6N_6O_2S$: C 31.86, H 2.67, N 37.15. Found: C 31.88, H 2.69, N 37.20.

5-(1-Methyl-4-nitro-1H-5-imidazolyl)-1H-1,2,4-triazol-3-amine (6)

Aminoguanidine hydrochloride (11 mmol) plus compound (10) (11 mmol) were heated in trifluoroacetic acid (5 mL) at 60° C for 15 h. The reaction medium was poured into ice-water (10 mL) and neutralized with saturated $NaHCO_3$ aqueous solution. The precipitate obtained was filtered, washed with water, and dried under vacuum. Compound (6) was obtained as yellow solid (60% yield), according to the procedure described above. MS (70 eV, *m/z*): 209 (M+, 92%), 193 (M+ -16, 48%), 179 (M+ -30, 22%), 125 (M+ -84, 100%); ^{1}H-NMR δ 3.86 (s, 3H, N-CH_3), 7.55 (sb, 2H, change with D_2O, NH_2), 7.81 (sb, 1H, change with D_2O, NH), 8.04 (s, 1H); ^{13}C-NMR δ 34.18 (N-CH_3), 128.14 (C4), 137.66 (C5), 142.00 (C2), 148.03 (C3*), 162.02 (C5*); Anal. calcd. for $C_6H_7N_7O_2$: C 34.45, H 3.37, N 46.88. Found: C 34.54, H 3.41, N 46.68.

5-(1-Methyl-4-amine-1H-5-imidazolyl)-1H-1,2,4-triazol-3-amine (7)

A mixture of 5 (76,84 mg, 0.34 mmol), 50 mg of 10% Pd/C, and 9.5 mL ethanol was hydrogenated at 1 atm for 1 h. The mixture was filtered in celite, the filtrate was washed with ethanol and concentrated. Compound (7) was obtained as yellow solid (98% yield). MS (70 eV, *m/z*): 196 (M+, 28%), 169 (M+ -27, 100%), 180 (M+ -16, 46%), 96 (M+ -100, 52%); ^{1}H-NMR δ 3.81 (s, 3H, N-CH_3), 7.22 (sb, 4H, change with D_2O, NH_2), 7.43 (sb, 4H, change with D_2O, NH_2), 8.10 (s, 1H); ^{13}C-NMR δ 34.01 (N-CH_3), 126.82 (C4), 136.00 (C5), 140.12 (C2), 147.85 (C2*) and 168.43 (C5*); Anal. calcd. for $C_6H_8N_6S$: C 36.72, H 4.11, N 42.83. Found: C 36.70, H 4.07, N 42.79.

5-(1-Methyl-4-amine-1H-5-imidazolyl)-1H-1,2,4-triazol-3-amine (8)

A mixture of 6 (71,06 mg, 0.34 mmol), 50 mg of 10% Pd/C, and 9.5 mL ethanol was hydrogenated at 1 atm for 1 h. The mixture was filtered in celite, the filtrate was washed with ethanol and concentrated. Compound (8) was obtained as yellow solid (100% yield), according to the procedure described above. MS (70 eV, *m/z*): 179 (M+, 100%), 163 (M+ -16, 22%), 152 (M+ -27, 68%), 97 (M+ -82, 43%); ^{1}H-NMR δ 4.21 (s, 3H, N-CH_3), 7.83 (sb, 2H, change with D_2O, NH_2), 8.30 (sb, 3H, change with D_2O, NH and NH_2), 8.01 (s, 1H); ^{13}C-NMR δ 33.52 (N-CH_3), 127.90 (C4), 137.41 (C5), 140.16 (C2), 146.99 (C3*), 161.80 (C5*); Anal. calcd. for $C_6H_9N_7$: C 40.22, H 5.06, N 54.72. Found: C 40.21, H, 5.04, N, 54.71.

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