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RESEARCH ARTICLE

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Cristina Northfleet de Albuquerque*
Laboratório de Síntese e Otimização de Fármacos, Departamento de Tecnologia Bioquímico-Farmacêtica, Faculdade de Ciências Farmacêuticas da Universidade de São Paulo
Av. Prof. Lineu Prestes, 580 bloco 16
São Paulo, SP, BRASIL
Email: northfle@usp.br

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A new synthetic route to obtaining megazol, an drug active in negligence's disease.

[1]Fabrício Arantes, [1]Hélio Lopes Júnior, [1]Sandra Moreto,
[1]Cristina Northfleet de Albuquerque*
[1]Laboratório de Síntese e Otimização de Fármacos, Departamento de Tecnologia Bioquímico-Farmacêtica, Faculdade de Ciências Farmacêuticas da Universidade de São Paulo, Av. Prof. Lineu Prestes, 580 bloco 16 São Paulo, SP, BRASIL.

Abstract

New drugs are urgently required for treatment of human African trypanosomiasis and Chagas´ disease. One compound with promise is megazol, a nitro heterocyclic compound that forms a nitro radical anion upon reduction. Megazol (2-amino-5-(1-methyl-5-nitro-2-imidazolyl)-1,3,4-thiadiazol) and related nitroimidazole compounds are being tested as antichagasic drugs. Little is known on the mode of action of megazol. However, there is evidence that 1-electron reduction of megazol to the corresponding nitro radical anion is a key step in the reaction mechanism.

It was observed that the obtaining of megazol can be made by different paths synthetic modifying procedures and reagents involved in the chemistry of heterocyclic particularly imidazoles. We evaluated experimental conditions, partial and overall income, as well as the characteristics of reagents involved in the processes indicated in one of the routes. Megazol was prepd. from 1-methylimidazole by cyanation, cyclization with thiosemicarbazide, nitration with concomitant acetylating of the amino group, and deacetylation.

Keywords: Synthesis, heterocyclic, imidazole, megazol.

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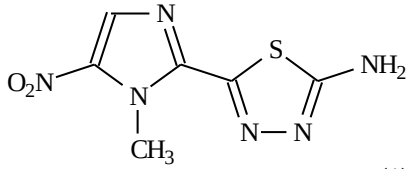
INTRODUCTION

The nitroimidazoles compounds are very interesting therapeutically [1,2,3]. We have many papers to describe their potentials in negligence's disease like amoebic, trichomonal, giardial and anaerobic bacterial infections [4,5]. However the certain nitroimidazoles are demonstrated in experimental animals of the mutagenic and carcinogenic properties [6, 7].

Nifurtimox and metronidazole are nitro heterocyclic that, like other nitro compounds, exhibit antibacterial, antiprotozoal and radiosensitizing properties[8]. These properties have been related to their electron affinity and more precisely to the reduction potential of the one-electron transfer pair ArNO2·/ArNO2 involved in the corresponding redox chain [9,10].

Derivative CL 64855 (2-amino-5-(1-methyl-5-nitro-2-imidazolyl)-1,3,4-thiadiazole) (figure 1), megazol, is a nitroimidazole that is very effective against *Trypanossoma cruzi*, a parasite responsible for Chagas' disease in South America [11,12,13,14]; the compound is of particular interest since it is active on all strains of the parasite. Owing to the rather low efficiency and severe side effects of nifurtimox and benznidazole, the only available drugs megazol represents a promising alternative. More recently it has been shown that this compound is also highly active against *Trypanosoma brucei* in association with suramin or melarsoprol[15,16].



(1)

Figure 1: structure of megazol.

Megazol (1), the chemical structure of interest in this work, was synthesized initially in 1968 for Asato and Berkelhammer using 5-nitroimidazol as a starting material. In years 80, brazilian researchers of the Foundation Institute Oswaldo Cruz and René Rachou Center, had related a great number of chemical substances active against the *Trypanosoma cruzi*, illness very common in Brazil. One of these substances, megazol, presented a great interest, as the tests "*in vivo*" in rats showed significant dressing effect, in only one dose. As a result of this biological test, more accurate studies of the analogous molecules had become essential, to determine the mechanism of action of this structure and its potential toxic effect. The synthesis route was the described by Albuquerque in 1999, and optimized by Novaes in 2011. In the present work we describe a new method to obtained megazol and analogous (figure 2).

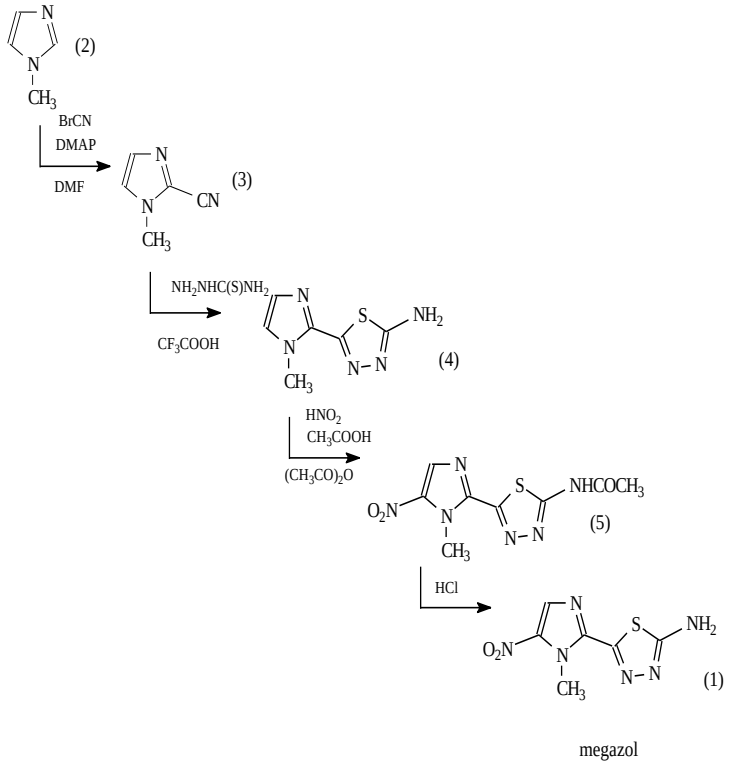


Figure 2: The new route to obtain megazol.

From the literature, we have developed a route different from the previously used to obtain megazol using procedures well known and simple implementation.

The route of synthesis was performed in four steps and the starting material 1-methylimidazole (2). The first stage of the route is the process of introduction a nitrile group in positon 2, after cyclization with thiosemicarbazone, nitration with a block amine and deblocks this amine group.

METHODS

All melting points were determined on open glass capillaries using an Electrothermal Marconi BTC 9090 melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on Perkin Elmer 1610 Model FTIR spectrophotometer using the KBr disc technique. ^{1}H NMR spectra and ^{13}C NMR spectra were recorded on Brucker Advance DPX-300 (300MHz ^{1}H and 75MHz ^{13}C) spectrometer (DMSO-d_6) and the chemical shift are given in δ (parts per million) downfield from tetramethylsilane (TMS) as an internal standard. The Mass Spectra (MS) were measured using a Nermag R1010 spectrometer. Reactions and column chromatographic separations were followed by layer chromatography using silica gel (with 254nm fluorescent indicator) on aluminium plates. DMF were dried by storing over 4A molecular sieves after distillation.

1-methyl-2-cyanoimidazole (3)

4-NN-dimethyl-aminopyridine (6.1g, 50mM) and cyanogens bromide (5.3g, 50mM) in DMF (20ml) under argon, at room temperature generate a yellow precipitate of cyanogens bromine dimethylamine pyridynium salt to which was added by syringe 1-methylimidazole (2) (1.7ml, 20mM) a 40ºC. After 20 hours stirring, the mixture was poured into a saturated sodium hydrogen carbonate solution (100ml) and extracted with dichloromethane (4x25ml). After evaporation of the organic phase and flash chromatography (CH_2Cl_2), 3 was obtained as a yellow crystals mp 260-5°C (dec.); (0.99g, 45%yield). ^{1}H NMR (300MHz, DMSO) δ (ppm): 3.49(s, 3H, NCH_3), 8.03(s, 1H, H5), 8.36 (s, 1H, H4). ^{13}C NMR (75 MHz, DMSO) δ (ppm): 30.7(NCH_3), 79.1 (CN), 130.9 (C2), 162.2 (C4 e C5). Mass spectrometry (E.I) m/z :108 (M^++1, 20%). IR (KBr): 3117 (NCH_3); 2235 (CN); 1508; 1471; 1411; 1394; 1286; 776 (imidazole ring).

1-methyl -2-(5-amino-1,3,4-thiadiazolyl)imidazole (4)

3 (0.55g, 5mM) mixed with thiosemicarbazine (0.5g, 5mM) was heated at 60°C in trifluoroacetic acid, overnight. The reaction mixture was poured in iced water (20ml) and was neutralized with sodium hydrogen carbonate. After recrystallization with ethanol we obtained 0.37g (2mM) of (3) yield 40%. mp 190°C; ^{1}H NMR (300 MHz, DMSO) δ (ppm): 3.96(s, 3H, NCH_3), 6.00(d, 1H, H4, J=1.5Hz), 7.3 (d, 1H, H5, J=1.5Hz). ^{13}C NMR (75 MHz, DMSO) δ (ppm): 34.7(NCH_3), 124.7(C5), 128.4(C4), 137.7(C2), 149.8 (C2'), 168.2(C5'). Mass spectrometry (E.I) m/z: 182 (M^++1, 20%). Anal. Calcd. for $C_6H_7N_5S$: C, 39.77; H, 3.89; N, 38.65 Found:C, 39.37; H, 3.73; N, 37.97 .

1-methyl-2-(5-acetamido-1,3,4-thiadiazolyl)-5-nitroimidazole (5)

4 (0.36g, 2mM) dissolved in acetic anhydride was added an in sulfonitric solution (2ml H_2SO_4 con., 2ml HNO_3 fuming) at 0°C. After half an hour the mixture was poured on crushed ice. The yellow precipitate was filtered washed with water (5ml), dried under vacuum, recrystalized with acetone-ethanol giving 5 (0.37g, 70%yield). mp 230-8°C ^{1}H NMR (300MHz, DMSO) δ (ppm): 2.24 (s, 3H, $COCH_3$); 4.39 (s, 3H, NH_3), 8.25(s, 1H, H4). ^{13}C NMR (75 MHz, DMSO): δ (ppm) 22.3 (CH_3CO), 35.2 (NCH_3), 133.1 (C4), 140.6 (C2), 141.4 (C5), 153.9 (CONH), 159.7 (C2'), 169.2 (C5'). Mass spectrometry (E.I) m/z: 270 (M^++1, 10%). IR (KBr): 3123(NH); 2917(CH); 1698(CONH); 1561; 1525; 1460; 1364; 1314; 1270 (imidazol and thiadiazol rings and nitro group).

1-methyl-2-(5-amino-1,3,4-thiadiazole)-5-nitroimidazole (1) Megazol

5 (0.36 g, 2 mM) was added to a chloride acid conc. The mixture is refluxed for 6h. After, the mixture was poured into ice (5mL). The yellow precipitate formed was filtered, washed with water (5 mL), after crystallisation in acetone and dried under vacuum, giving 5 (0.21 g, 70%). MP 270°C; ^{1}H NMR (3000 MHz, DMSO) δ (ppm): 4.32 (s, 3H, NCH_3), 7.8 (b, 2H, NH_2), 8.2 (s, 1H, H4)). ^{13}C NMR (75 MHz, DMSO) δ (ppm): 35.0 (s, NCH_3), 133.1 (C4), 140.1 (C5), 141.4 (C2), 48.2 (C2'), 169.9 (C5'). Mass spectrometry (EI) m/z 227 (M^++1, 80%) Anal.Calcd for $C_6H_6N_6O_2S$: C,31.83 ; H,2.67 ; N,37.15 Found : C,31.64 ; H,2.62 ; N,36.41.

RESULTS:

On the table 1 shows the partial yields of each step of the synthesis route proposal.

Step	Process	Reagent	Time	Temperature	Yield (%)
1	Cyanation	BrCN/DMAP	20h	40ºC	45%
2	Cyclization	Thiosemicarbazine/ trifluoroacetic acid	12h	60°C	40%
3	Nitration	Acetic anhydride/ sulfonitric solution2ml H_2SO_4 con., 2ml HNO_3 fuming	30 min.	0°C	70%
4	Deblocking of the amine group	HCl 2N	6h	Reflux	70%

Table 1: summary of the yields of each process of the synthesis route proposed in this study.

DISCUSSION:

The first step is to match the product of 1-metilimidazole (2). This reaction employs up to the cyanogens bromide, to introduction of a nitrile group in 2-position of imidazol (3).

A direct method for obtaining 2-cyanoimidazoles, which is a complex formed between the cyanogens bromide and 1.4-dimethylaminopyridine in dimethylformamide, resulting in salt bromide 1-cyano-4-dimethylaminopyridine (CAP)[18,19,20], according to the outlined below in figure 3:

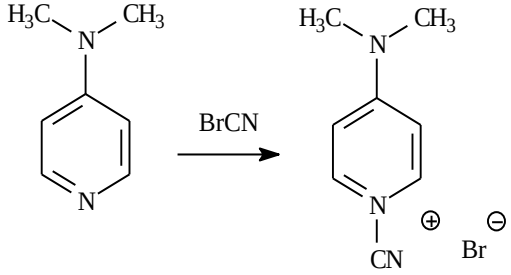


Figure 3: Complex CAP[18, 19, 20].

This conversion is characterized by the formation of the electrophilic species NC^+. Get up, then the 1-methyl-2-cyanoimidazol, with an average yield of 45%, as the outlined below in figure 4:

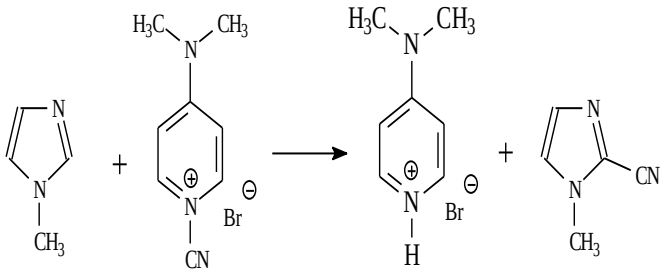


Figure 4: Mechanism proposed to reaction of 1-metilimidazol by CAP[18, 19, 20].

The functionalization of 1-metilimidazol by nitrile group in position 2 was obtained in the previous step is the key to the stage of cyclization. It is through the group that thiosemicarbazide, in the presence of trifluoracetic acid will allow the formation of the thiadiazolic ring (3)[14].

The second step is based in the method proposed by Albright & Shepherd in 1973, the cyclization with thiosemicarbazide in trifluoracetic acid is performed with the 5-nitro-1-methyl-2-cyanoimidazol. In the new proposed route the 1-methyl-2-cyanideimidazol react using the same principle, but in the absence of nitro group in position 5 of the imidazol ring. According to literature, the nitro group activates the imidazol ring. In this case, the nitro group brings different results with regard to income and the time of reaction[14].

However, the use of a strong acid (trifluoracetic acid) favors the cyclization due to the facility of the exit of ammonium group and their neutralization. We obtained 40%.

Whereas the trifluoroacetic acid is a strong acid and in this case used as the reaction solvent, it is believed that there was a decomposition of 1-methyl-2-cyanoimidazol, since the yield was lower.

The third and fourth stages of the route under study are related to the process of nitration of the ring imidazol. This step was based on the route proposed by Remmers, Gibs and Weiss in 1969 [21], where the amine ring linked to 1,3,4-thiadiazol is initially protected by the action of acetic anhydride and, after it the nitric acid is added, and then unprotected employing up hydrochloric acid solution.

In this case, the nitration should be initiated only after the protection of the amino group. The protection is done using the acetic anhydride which is also used as a solvent. The group will be converted to amine amide. We obtained 70%.

We then started testing for the release of amide and obtaining end of megazol. This process was based on a U.S. Patent[22] and reflux indicates that a simple product of acetylated with hydrochloric acid 1N by 2h is sufficient to hydrolyze the group acetate, giving a yield of 80%.

However, in the megazol acetylated place in the presence of concentrated hydrochloric acid reflux in by 6h. In this case we had a promising result with a yield of 70%.

CONCLUSION:

From these results we achieved the goals since the initial proposed route was experimentally tested giving significant results.

In our experiments had an overall income of 26% still low, but substantial compared with other routes indicated in the literature.

Our experiments showed the feasibility of the processes involved in the proposed route which are: the cyanation of 1-metilimidazol (45%) (2); cyclization and the formation of the ring 1,3,4-thiadiazol from the 1-methyl - 2-cyanoimidazol with thiosemicarbazine (40%) (3), the nitration of 1-methyl-2-yl-5-amino-(1,3,4-thiadiazolil) imidazole (70%) (4) and the release of the amine and training of megazol by the hydrolysis with concentrated hydrochloric acid (70%).

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