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Synthesis, spectrometric characterization and trypanocidal activity of some 1,3,4-thiadiazolines derivatives

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ABSTRACT

Six 1,3,4-thiadiazolines derivatives were synthesized by cyclization of thiosemicarbazones under acetylating condition with yields going from 27 to 94%. The products purity was confirmed by LC/MS (Mass Spectrometry Coupled with High-Performance Liquid Chromatography) and they were characterized using spectrometry IR, NMR ^{1}H and ^{13}C (Nuclear Magnetic Resonance). These compounds were then tested *in vitro* on *Trypanosoma brucei brucei* according to the "LILIT, Alamar Blue" method to estimate their trypanocidal activity. 1,3,4-thiadiazoline **6** (IC_{50} = 38,79 µM) was the most active of all compounds.

Keywords: Cyclization, thiosemicarbazones, LC/MS, NMR, IR, *Trypanosoma brucei brucei.*

INTRODUCTION

African trypanosomes are parasitic protozoa that affect both man and animals. *Trypanosoma brucei brucei* is one of the causative agents of "Nagana" which decimate cattle. *Trypanosoma brucei gambiense and Trypanosoma brucei rhodesiense* are responsible for the African human trypanosomiase, an endemic disease in sub-Saharan Africa with nearly 50.000 estimated cases and a population at risk of 60 million people (WHO, 2007a). The toxicity and adverse effects of drugs that have been commonly used to treat this disease, their impractical dosing regimens (WHO, 2007b) as well as the damage caused in the sector of the breeding require the development of new active molecules in a most safe chemotherapeutic approach. Accordingly, our interest have been focused on 1,3,4-thiadiazolines and 1,3,4-thiadiazoles which showed during the 50 last years a broad spectrum of pharmacological properties.

Indeed, these small molecules exhibit various biological activities such as antituberculosis (Shucla et al.,1984; Foroumadi et al., 2002), antiviral (García et al., 2003), anticonvulsant (Chapleo et al., 1988), Fungicidal (Hagiwara et al., 1992), antihypertensive (Mazzone et al., 1993), hypoglycemic (Hanna et al., 1993), antimicrobial (Mamolo et al., 1996) anti-inflammatory (Labanauskas et al., 2001), adenosine A3 receptor antagonists (Jung et al., 2004) and anticancer activities (Chou et al., 2003; Nakai et al., 2009). Finally, 1,3,4-Thiadiazole and related compounds are of great interest in chemistry owing to their bioactivity on certain plant growth regulating effects as well as antimicrobial activity (Sancak et al., 2007).

However, the literature does not set great store by the trypanocidal activity of 1,3,4-thiadiazolines. The aim of this work is to synthesize some 1,3,4-thiadiazolines and to estimate their trypanocidal activities.

MATERIALS AND METHODES

Chemistry

Apparatus

We used thin layer chromatography (TLC) to estimate the compounds purity, to follow the evolution of the reactional medium of 1,3,4-thiadiazolines, and then to achieve their purification on silica gel column. The solvent used is the mixture of dichloromethane/ethyl acetate (2/1) or dichloromethane/methanol (9/1). Compounds purity was confirmed by LC/MS. The melting points were taken on a fusionometer *eletrothermal 1A 9000.* The spectrometric data were recorded with the following instruments: IR, Perkin Elmer FTIR 286; H NMR 1 and 13 NMR, Bruker 400; LC/MS in APCI mode on a C18 column. 1,3,4-thiadiazolines are synthesized as follows:

Synthesis of 1,3,4-thiadiazolines

A mixture of ketone (20 mmol dissolved in 100 ml of ethanol) and thiosemicarbazide (20 mmol dissolved in 20 ml of 1N hydrochloric acid) is stirred until the thiosemicarbazone precipitates. The precipitate is filtered, washed, dried and then recrystallized in ethanol (96 °C) to give purified thiosemicarbazone crystals.

This thiosemicarbazone (0.25 mmol) was dissolved in 0.5 mL of pyridine and 0.5 mL of acetic anhydride and the mixture was heated at 110 °C during 3 h with magnetic stirring to give the 1,3,4- thiadiazoline derivative (Brousse et al., 2002) which is filtered and purified by flash chromatography (Figure 1).

Antitrypanosomal activity (LILIT, Alamar Blue^{TM})

The test is performed on the bloodstream form of the strain 427 of *Trypanosoma brucei brucei* by the "Lilit Alamar Blue" method (Baltz et al., 1985; Räz et al., 1997). The stock solutions of 1,3,4-thiadiazolines have been prepared from an initial concentration of 10 mg/ml in DMSO. The trypanosomes are grown in a medium containing 10% of heat-inactivated fetal calf serum and bloodstream form supporting factor. The trypanosome suspensions were adjusted to 5.10^{-4} tryp/mL. In each well, 50 µl of different dilutions of the stock solution were added to 50 µl of suspension of trypanosomes. The plates were then incubated at 37 °C for 72 hours in an atmosphere with 5% CO_2. 10 µl of dye "Alamar Blue^{TM}" is added to each well and then incubated for 4 hours.

The dye "Alamar Blue^{TM}" is a reagent for detecting enzymatic activity. The wells in which the concentration of compound is insufficient to inhibit the proliferation of trypanosomes are stained. The CMI is the concentration of unstained wells in which there is the lowest amount of 1,3,4-thiadiazole. The plate reading is made in comparison with control wells on a fluorescence plate reader using an excitation wavelength of 530 nm and an emission wavelength 590 nm.

Statistical analysis

Lethality assays were evaluated by Excel computer statistical program to determine the LC_{50}. Values are means of two experiments; standard deviation (± sd) is given in parentheses (Table 1).

RESULTS

Chemistry

Six 1,3,4-thiadiazolines were synthesized with yields going from 27 to 94%. The physical and spectrometric data of these compounds are reported below.

5-Acetamido-3-*N*-acetyl-2-methyl-2-phenyl-1,3,4-thiadiazoline (1)

Yield: 57%. M.p : 224-225 °C. Rf $_{CH2Cl2/MeOH\ (9/1)}$: 0.53: MS: $[MH]^+$cal 278,09564 $[MH]^+$found 278.09577 IR data (KBr cm^{-1}): 3145 ν(NH), 1694, 1631, 1615 ν(C=O amide). ^1H NMR data ($CDCl_3$ δ ppm): 1.84 (3H, s, CH_3); 2.22 (3H, s, CH_3 amide); 2.29 (3H, s, CH_3 amide); 7.15-7.35 (5H, several signals, ArH); 9.14(1H, s, NH). ^{13}C NMR data ($CDCl_3$ δ ppm): 22.87 (CH_3); 25.89 and 26.86 (CH_3 amide); 80.03 (C_2 in the ring); 124.99-142.82 (aromatic C); 143.48 (C=N); 168.84 and 169.27 (C=O amide).

5-Acetamido-3-*N*-acetyl-2-(2´-chlorophenyl)-2-methyl-1,3,4-thiadiazoline (2)

Yield: 27%. M.p : 215-217 °C. Rf $_{CH2Cl2/AcOET\ (2/1)}$: 0.58 MS: $[MH]^+$cal 312.0568 $[MH]^+$found 312.0565. IR data (KBr cm^{-1}): 3160 ν(NH), 1698, 1644, 1611 ν(C=O amide). ^1H NMR data ($CDCl_3$ δ ppm): 1.86 (3H, s, CH_3); 2.26 (3H, s, CH_3 amide); 2.36 (3H, s, CH_3 amide); 7.19-7.42 (4H, several signals, ArH); 9.61 (1H, s, NH). ^{13}C NMR data ($CDCl_3$ δ ppm): 22.96 (CH_3 amide); 23.01 (CH_3 amide); 28.85 (CH_3); 78.35 (C_2 in the ring); 126.64-137.27 (aromatic C); 144,37 (C=N); 168.68 and 168.78 (C=O amide).

5-Acetamido-3-*N*-acetyl-2-(4´-chlorophenyl)-2-methyl-1,3,4-thiadiazoline (3)

Yield: 58%. M.p : 214-216 °C. Rf $_{CH2Cl2/AcOET\ (2/1)}$: 0.61 MS: $[MH]^+$cal 312.0568 $[MH]^+$found 312.0567. IR data (KBr cm^{-1}): 3146 ν(NH), 1694, 1633, 1617 ν(C=O amide). ^1H NMR data ($CDCl_3$ δ ppm): 1.75 (3H, s, CH_3); 2.22 (3H, s, CH_3 amide); 2.24 (3H, s, CH_3 amide); 7.19-7.27 (4H, several signals, ArH); 10.14 (1H, s, NH). ^{13}C NMR. data ($CDCl_3$ δ ppm): 22.49 (CH_3); 23.78 (CH_3 amide); 26.62 (CH_3 amide); 78.90 (C_2 in the ring); 126.68-141.31 (aromatic C); 144.86 (C=N); 169.40 and 169.56 (C=O amide).

5-Acetamido-3-*N*-acetyl-2-(3´-bromophenyl)-2-methyl-1,3,4-thiadiazoline (4)

Yield: 76%. M.p : 238-239 °C. Rf $_{CH2Cl2/AcOET\ (2/1)}$: 0.57 MS: $[MH]^+$cal 358.0048 $[MH]^+$found 358.0046. IR data (KBr cm^{-1}): 3148 ν(NH), 1695, 1614 ν(C=O amide). ^1H NMR (DMSO δ ppm): 2.03 (3H, s, CH_3); 2.20 (3H, s, CH_3 amide); 2.27 (3H, s, CH_3 amide); 7.26-7.52 (4H, several signals, ArH); 11.69 (1H, s, NH). ^{13}C NMR data (DMSO δ ppm): 22.40 (CH_3); 23.58 (CH_3 amide); 26.30 (CH_3 amide); 77.86 (C_2 in the ring); 121.72-142.30 (aromatic C); 144.36 (C=N); 167.77 and 169.45 (C=O amide).

5-Acetamido-3-*N*-acetyl-2-(4´-bromophenyl)-2-methyl-1,3,4-thiadiazoline (5)

Yield: 78%. M.p : 211-213 °C. Rf $_{CH2Cl2/AcOET\ (2/1)}$: 0.67 MS: $[MH]^+$cal 358.0048 $[MH]^+$found 358.0038. IR data (KBr cm^{-1}): 3218, 3148 ν(NH), 1693, 1614 ν(C=O amide). ^1H NMR data ($CDCl_3$ δ ppm): 1.75 (3H, s, CH_3); 2. 22 (3H, s, CH_3 amide); 2.24 (3H, s, CH_3 amide);7.19-7.24 (4H, several signals, ArH); 10.32 (1H, s, NH). ^{13}C NMR data ($CDCl_3$ δ ppm): 22.54 (CH_3); 23.85 (CH_3 amide); 26.59 (CH_3 amide); 78.85 (C_2 in the ring); 121.93-141.85 (aromatic C); 144.34 (C=N); 169.39 and 169.55 (C=O amide).

5-Acetamido-3-*N*-acetyl-2-(2´-nitrophenyl)-1,3,4-thiadiazoline (6)

Yield: 94%. M.p : 245-246 °C. Rf $_{CH2Cl2/AcOET\ (2/1)}$: 0.37 MS: $[MH]^+$cal 308.3130 $[MH]^+$found 308.3132. IR data (KBr cm^{-1}): 3232, 3192 ν(NH), 1682, 1664 ν(C=O amide). ^1H NMR data (DMSO δ ppm): 2.04 (3H, s, CH_3 amide); 2.26 (3H, s,

5-Acetamido-3-*N*-acetyl-2-(2´-nitrophenyl)-1,3,4-thiadiazoline (6)

Yield: 94%. M.p : 245-246 °C. Rf $_{CH2Cl2/AcOET\ (2/1)}$: 0.37 MS: $[MH]^+$cal 308.3130 $[MH]^+$found 308.3132. IR data (KBr cm^{-1}): 3232, 3192 ν(NH), 1682, 1664 ν(C=O amide). ^{1}H NMR data (DMSO δ ppm): 2.04 (3H, s, CH_3 amide); 2.26 (3H, s, CH_3 amide); 5.51 (1H, s, C_2 in the ring); 7.26-8.18 (4H, several signals, ArH); 11.86 (1H, s, NH). ^{13}C NMR data (DMSO δ ppm): 21.70 (CH_3); 22.41 (CH3 amide); 63.15 (C_2 in the ring); 125.52-145.83 (aromatic C); 146.31 (C=N) and 169.66 (C=O amide).

IR spectra of 1,3,4-thiadiazolines show bands in the area of 3232-3145 cm-1 due to the stretching vibration of NH. In ^{1}H NMR, the most deshielded proton, which is linked to the central nitrogen atom, appears as a broadened singlet between 8.9 and 11.69 ppm. Ring closure in 1,3,4-thiadiazolines may be observed by: 1) the disappearance of the signal between 176 and 180 corresponding to the thiocarbonyl; 2) the appearance of a signal between 77 and 81 ppm assigned to C-2; and 3) the signals of the carbonyl and methyl moieties of the acetyl groups incorporated to the molecule.

In mass spectrometry, the $[MH]^+$ peaks obtained in APCI mode correspond to molecular weights expected for all products. In LC mode, all 1,3,4-thiadiazoles have a single peak confirming their purity.

Trypanocidal activity

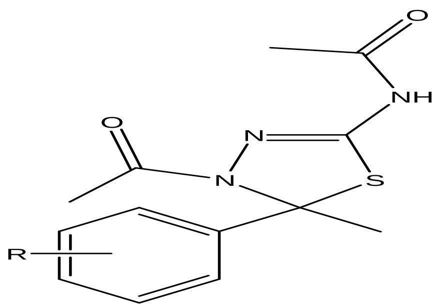
The synthesized compounds were tested for their trypanocidal activity on *Trypanosoma brucei brucei*. The test results are reported in Table 1.

The data of this table indicate that compounds 1 and 4 have LC_{50} values well above 100 µM.

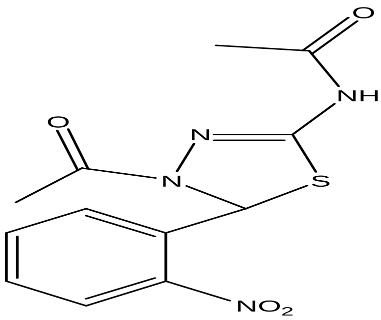
The thiadiazoline 2, 3 and 5 have respective LC_{50} values of 172.76, 175.93 and 134.70 µM. LC_{50} values are greater than 100 µM for thiadiazoline 1-5 which have halogen substituents (Cl or Br) on the benzene ring. These compounds have a little trypanocidal activity. The thiadiazoline 6 which have a nitro group in ortho position on the benzene ring appears as a moderate trypanocidal with a LC_{50} value of 38.79 µM.

Table 1: Structure and trypanocidal activity of 1,3,4-thiadiazolines.

Compounds	R	IC_{50}-moy ± sd (µg/ml)	IC_{50}-moy ± sd (µM)
1	-	>100	-
2	2’-Cl	53,73 ± 3,1	172,76 ± 8,44
3	4’-Cl	54,72 ± 0,6	175,93 ± 1,83
4	3’-Br	>100	-
5	4’-Br	47,96 ± 4,94	134,7 ± 16,63
6	-	11,96 ± 4,26	38,79 ± 13,81



1 -5



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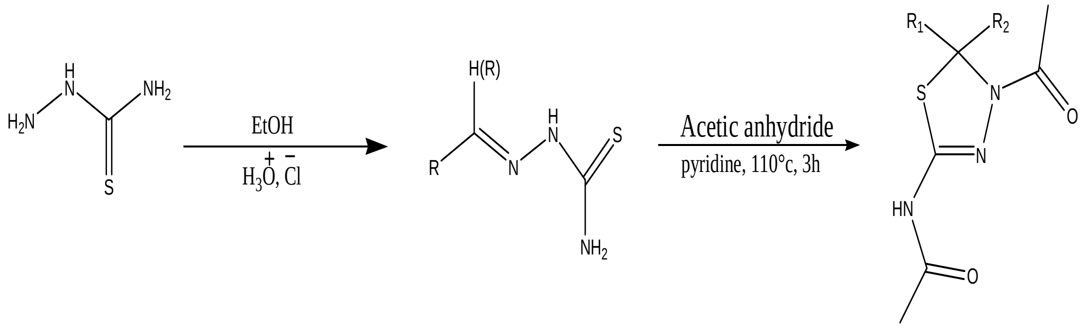

Figure 1: Synthesis of 1,3,4-thiadiazolines.

DISCUSSION

The spectrometric data of the six synthesized compounds are in conformity with the structures suggested for the products: 5-Acetamido-3-*N*-acetyl-2-methyl-2-phenyl-1,3,4-thiadiazoline (**1**); 5-Acetamido-3-*N*-acetyl-2-(2´-chlorophenyl)-2-methyl-1,3,4-thiadiazoline (**2**); 5-Acetamido-3-*N*-acetyl-2-(4´-chlorophenyl)-2-methyl-1,3,4-thiadiazoline (**3**); 5-Acetamido-3-*N*-acetyl-2-(3´-bromophenyl)-2-methyl-1,3,4-thiadiazoline (**4**); 5-Acetamido-3-*N*-acetyl-2-(4´-bromophenyl)-2-methyl-1,3,4-thiadiazoline (**5**); 5-Acetamido-3-*N*-acetyl-2-(2´-nitrophenyl)-1,3,4-thiadiazoline (**6**).

Thiosemicarbazones are known to inhibit cysteine proteases such as cathepsins : TbcatB and rhodesain. It has been suggested that these proteases may be involved in nutrient acquisition, degradation of host proteins, evasion of the host immune response, or crossing of the blood brain barrier and are essential for the parasite survival (Caffrey et al., 2001; Hirumi et al., 1994). Inhibition of these proteases by thiosemicarbazones has been demonstrated and would consequently result in the death of the parasite *Trypanosoma brucei brucei* (Caffrey et al., 2001; Hirumi et al., 1994). 1,3,4-thiadiazolines which are cyclic deravatives of thiosemicarbazones could have the same action mechanism on *Trypanosoma brucei brucei.* The literature does not set great store by the trypanocidal activity of 1,3,4-thiadiazolines. Then, cyclized products were purified by flash chromatography and their purity confirmed by LC/MS to highlight the specific trypanocidal activity of 1,3,4-thiadiazolines. This cyclized compounds showed trypanocidal activity. Thus, IC_{50} value of compound **6** (38.79 µM) indicates that this compound is the most active. The other 1,3,4-thiadiazolines have IC_{50} value higher than 100 µM. According to the work of Du et al. (2002) and Fujii et al. (2005), thiosemicarbazones are trypanocidal when their IC_{50} values are less than 10 µM, moderate trypanocidal if these values are between 10 and 100 µM, and have little or no activity when their IC_{50} are higher than 100 µM.

Conclusion

In order to estimate their trypanocidal activity, six 1,3,4-thiadiazolines : 5-Acetamido-3-*N*-acetyl-2-methyl-2-phenyl-1,3,4-thiadiazoline (**1**); 5-Acetamido-3-*N*-acetyl-2-(2´-chlorophenyl)-2-methyl-1,3,4-thiadiazoline (**2**); 5-Acetamido-3-*N*-acetyl-2-(4´-chlorophenyl)-2-methyl-1,3,4-thiadiazoline (**3**); 5-Acetamido-3-*N*-acetyl-2-(3´-bromophenyl)-2-methyl-1,3,4-thiadiazoline (**4**); 5-Acetamido-3-*N*-acetyl-2-(4´-bromophenyl)-2-methyl-1,3,4-thiadiazoline (**5**) and 5-Acetamido-3-*N*-acetyl-2-(2´-nitrophenyl)-1,3,4-thiadiazoline (**6**) were synthesized and purified by flash chromatography. Their purity was confirmed by LC/MS and their structures were completely determined by spectrometric analysis. All compounds were tested on *Trypanosoma brucei brucei*. The 1,3,4-thiadiazoline (**6**) was found more active than the other compounds. This first study lead us to conclude that 1,3,4-thiadiazolines may have trypanocidal activity.

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