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Research Article

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Benzaldehyde and Derivatives N(4)-Phenyl-3-Thiosemicarbazones: Synthesis, Characterization and Biological Activity

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Abstract A class of small molecules, thiosemicarbazones and derivatives have been studied over the last few years due to their wide pharmacological versatility. Due to having this wide-spectrum biological activity, interest on these compounds has been considerably increased in the pharmaceutical sector at the present time. Here, benzaldehyde and derivatives were used on N(4)-phenyl-3-thiosemicarbazide with glacial acetic acid to synthesize four thiosemicarbazones corresponding (**1-4**) in good yields (64-85%). Structures of compounds were characterized by spectrometric alanalysis: FT-IR, NMR ^{1}H & ^{13}C and MS spectra. A theoretical study based on their chemical structure has been made. *In vitro* antiparasitic activity of products has been evaluated on the on the bloodstream form of the strain 427 of *Trypanosoma bruceibrucei*. All compounds presented physical properties compatible with reasonable pharmacokinetics and drug availability, but they showed a low half inhibitory concentration (IC_{50}> 100 µM) and then were not actives on the trypanosomes tested. Other biological activity would be explored on other pathogens.

Keywords Synthesis, N(4)-phenyl-3-thiosemicarbazones, reasonable pharmacokinetics

Introduction

Past decades, mainly thiosemicarbazones have exposed class of compounds have multi-donor ligands still growing attracts in the research group [1]. Thiosemicarbazones, as well as their metal complexes formed the subject of great interest to many researchers for a number of years. Apart from their diverse chemical and structural characteristics, the interest on these compounds also stems from their wide spectrum of biological activity [2-4]. Recently, due to having this wide-spectrum biological activity, interest on these compounds has been considerably increased in the pharmaceutical sector at the present time [5-7]. Thiosemicarbazones were suggested as pesticides, fungicides and catalysts in chemical and petro-chemical processes [8]; they were studied for the activity against tuberculosis, leprosy, psoriasis, rheumatisms, trypanosomiasis and coccidiosis [9]. They represent validated drug leads that kill

several species of protozoan parasites through the inhibition of cysteine proteases as well as other novel targets [10]. They were used in the analysis of metals for device applications relative to telecommunications, optical computing, optical storage and optical information processing [11]. The reasons for such pharmacological activities were due to the ability of thiosemicarbazones to chelate strongly with transition metal ions in biological systems binding through thio-keto sulphur and hydrazine nitrogen atoms [3].

In the light of this important data which have been achieved with the literature survey considering that the thiosemicarbazones are biologically active compounds, synthesis of the thiosemicarbazone derivatives expected to show positive activity was carried out. In this work, we have synthesized N(4)-phenyl-3-thiosemicarbazones of benzaldehyde and its substituted derivatives with chlorine (in ortho) and the group methoxy (in ortho and para positions). The anti-parasiticactivity on *Trypanosoma bruceibrucei* of the compounds was evaluated. This parasite causes Animal African Trypanosomiasis (AAT) which is a major constraint for the livestock industry and public health in developing countries [12-13].

Materials and Methods

Equipment

Their melting points were taken on a *fusionometer* of the type *electrothermal1A 9000* and are not corrected.

The infrared (IR) spectra of synthetic compounds were recorded on an apparatus *Perkin-Elmer FT-IR* 286. The frequencies of absorption bands are expressed in cm^{-1}.

The nuclear magnetic resonance (NMR) spectra were registered on a spectrophotometer type *Brucker*400 in DMSO-d_6 or $CDCl_3$ and the frequencies for ^1H and ^{13}C are 400.130 MHz and 100.612 MHz respectively. Chemical shifts are given in parts per million (ppm) relative to tetramethylsilane as internal standard. Multiplicity is designated as singlet (s) and multiplet (m).

Mass spectrometry (MS) data were reported using a LCQ advantage mass spectrometer with a source at atmospheric pressure chemical ionization (APCI), mode [MH^+].

Reagents

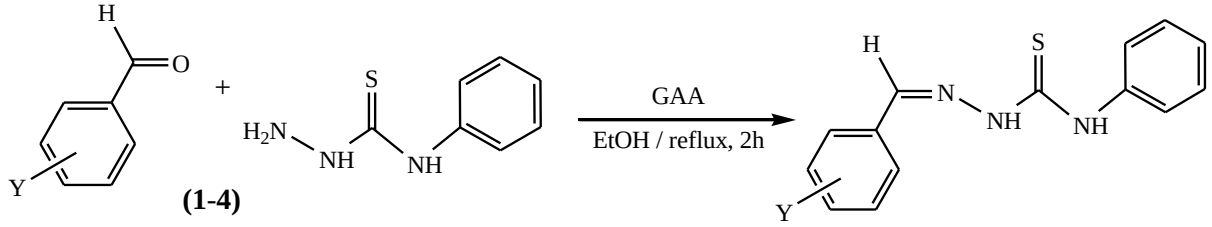
The 4-phenyl-3-thiosemicarbazide was obtained from ^{A}ALDRICH^R and used on benzaldehyde, *o*-chloro benzaldehyde, *o*-methoxybenzaldehyde and *p*-methoxybenzaldehyde purchased from Accros Organic, Janssen Chimica and Fluka AG-Buchs SG. The glacial acetic acid (AAG) used in the reactions is obtained from PROLABO. All reagents were used in the work without other purifying.

Compounds were synthesized via the following synthesis route (**scheme 1**).

Methods

Synthesis: general protocol for the preparation of the compounds

A solution of 4-phenyl-3-thiosemicarbazide (1 mmol) in ethanol (1 mL) was added slowly to a stirring solution of appropriate aldehyde (1 mmol) in 0.5 mL of ethanol (EtOH) containing (0.2 mL) of glacial acetic acid (GAA). The solution was heated to reflux, on a versatile oil bath, monitoring in TLC, for 2 hours and solutions were cooled. The precipitate obtained was filtered and recrystallized from ethanol (95°) to give desired product.



(1-4)

1: Y = H ; **2** : Y = *o*-Cl (ortho); **3** : Y = *o*-OCH_3 (ortho) ; **4** : Y = *p*-OCH_3 (para)

Scheme 1: Synthetic routes of 4-phenyl-3-thiosemicarbazones (scaffold)

The structure of each product was characterized with different spectrometric analyses to confirm the synthesis of products. After this step, all compounds have been submitted to the *in vitro* anti-trypanosomal activity on the bloodstream form of the strain 427 of *Trypanosoma bruceibrucei* followed biological methods.

Pharmacology

Anti-trypanosomal activity

The assessment is performed on the bloodstream form of the strain 427 of *Trypanosoma brucei brucei* by the «LILIT AlamarBlue^{TM}» method [14-16]. The stock solutions of each thiosemicarbazone have been prepared from an initial concentration of 10 mg/mL^{-1} 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 $5x10^4$ tryp.mL^{-1}. 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 "AlamarBlue^{TM}" is added to each well and then incubated for 4 hours. The dye "AlamarBlue^{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 MIC is the concentration of unstained wells in which there is the lowest amount of thiosemicarbazone. 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.

Results and Discussion

Chemistry

We have synthesized four N(4)-phenyl-substituted thiosemicarbazones such as: benzaldehyde 4-phenyl-3-thiosemicarbazone (**1**), *o*-chlorobenzaldehyde 4-phenyl-3-thiosemicarbazone (**2**), *o*-methoxybenzaldehyde 4-phenyl-3-thiosemicarbazone (**3**) and *p*-methoxybenzaldehyde 4-phenyl-3-thiosemicarbazone (**4**). Spectrometrical methods analysis: IR, NMR ^{1}H & ^{13}C and MS were used to characterize the structure of each molecule.

Characterization of synthetic compounds

Benzaldehyde 4-phenyl-3-thiosemicarbazone (**1**)

Yield: 85%; m.p: 199-200 °C; **IR** (NaCl, ν in cm^{-1}): broad 2989 ν(NH), 1590 ν(C=N)1227 ν(C=S); **^{13}C NMR** (DMSO-$_{d6}$, δ in ppm): 176.00 (C=S), 142.83 (C=N), 139.04, 133.99, 130.01, 128.62, 127.61, 125.91, 125.31 (C aromatics); **^{1}H NMR** ($CDCl_3$, δ in ppm): 10.25 (s, 1H, =NNH-), 9.20 (s, 1H, CSNH-Ph), 7.97 (s, 1H, HC=N), from 7.65 to 7.25 (m, 10H aromatics); **MS** (m/z): [MH$^+$] 256.02; [MH$^+$] theoretical 256.08.

o-chlorobenzaldehyde 4-phenyl-3-thiosemicarbazone (**2**)

Yield: 75%; m.p: 198-199°C; **IR** (NaCl, ν in cm^{-1}): broad 2999 ν(NH), 1598 ν(C=N), 1258 ν(C=S); **^{13}C NMR** (DMSO-$_{d6}$, δ in ppm): 176.24 (C=S), 138.96 (C=N), 138.78 , 133.26 , 131.36 , 129.73 , 128.06 , 127.81 , 127.28 , 126.05 , 125.47 (C aromatics) ; **^{1}H NMR** ($CDCl_3$, δ in ppm): 9.60 (s, 1H, =NNH-), 9.15 (s, 1H, CSNH-Ph), 8.25 (s, 1H, HC=N) , from 7.95 to 7.25 (m, 9H aromatics); **MS** (m/z): [MH$^+$] 290.07; [MH$^+$] theoretical 290.04.

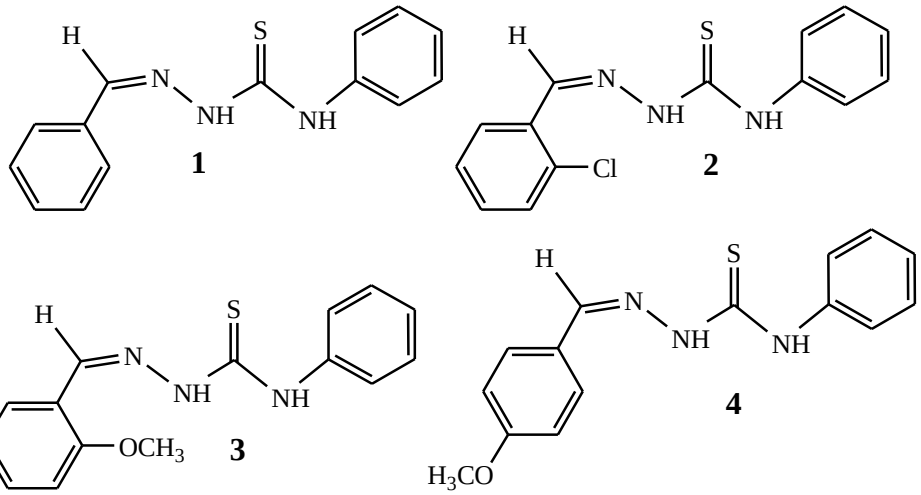
o-methoxybenzaldehyde 4-phenyl-3-thiosemicarbazone (**3**)

Yield: 64%; m.p: 158-159°C; **IR** (NaCl, ν in cm^{-1}): 3294, 3213 ν(NH), 1595 ν(C=N), 1253ν(C=S and C-O-C); **^{13}C NMR** ($CDCl_3$, δ in ppm): 175.77(C=S), 139.11 (C=N), 158.49, 137.95 , 133.55, 132.14, 128.80, 126.22, 126.10, 124.32, 121.48, 120.88, 111.27 (C aromatics), 55.65 (O-CH_3); **^{1}H NMR** ($CDCl_3$, δ in ppm): 9.35 (s, 1H, =NNH-), 9.20 (s, 1H, CSNH-Ph), 8.30 (s, 1H, HC=N), from 7.90 to 6.90 (m, 9H aromatics), 3.85 (s, 3H, H_3C-O); **MS** (m/z): [MH$^+$] 286.05 ; [MH$^+$] theoretical 286.09.

p-methoxybenzaldehyde 4-phenyl-3-thiosemicarbazone (**4**)

Yield: 73%; m.p: 178-179°C ; **IR** (NaCl, ν in cm^{-1}): broad 3146 & 2985 ν(NH), 1608 ν(C=N), 1249.30 ν(C=S and C-O-C); **^{13}C NMR** ($CDCl_3$, δ in ppm): 175.63 (C=S), 145.78 (C=N), 161.81, 137.96, 129.14, 128.83, 126.16, 125.53, 124.46, 114.46 (C aromatics), 55.47(O-CH_3); **^{1}H NMR** ($CDCl_3$, δ in ppm): 9.55 (s, 1H, =NNH-), 9.25 (s, 1H, CSNH-Ph), 7.90 (s, 1H, HC=N), from 7.70 to 6.95 (m, 9H aromatics), 3.85 (s, 3H, H_3C-O); **MS** (m/z): [MH$^+$] 286.05; [MH$^+$] theoretical 286.09

Structure of synthesized compounds



Analyses of spectrometrical data presented the following results:

In **IR spectra**, the vibrations of typical bands -NH-are observed between (broad) 3294 and 2985 cm^{-1}, C=N and C=S bands: 1608-1595 and 1258-1227 cm^{-1} respectively in each molecule; we note also C-O-C bands at 1253 and 1249 cm^{-1}, in products **3** and **4**.

In **^{13}C NMR** spectra, peaks of C=S are shown from 176.24 to 175.63 ppm and C=N between 145.78-138.96 ppm in all molecules, peaks of O-CH_3 appear at 55.65 and 55.47 ppm in products **3** and **4** respectively. All aromatics carbons of compounds were appeared in the range from 161.81 to 111.25 ppm.

^{1}H NMR spectra give the characteristics protons existing in each structure: signals of protons (=NN**H**-) were identified between 10.25-9.35 ppm, protons in (CSN**H**-Ph) appear from 9.25 to 9.15 ppm and the signal of the typical proton **H**C=N is shown in the range of 8.30 to 7.90 ppm. Aromatics protons were obtained between 7.95-6.95 ppm in the compounds. Protons of methoxy group (O-C**H**$_3$) are shown in products **3** and **4** at 3.85.

MS spectra give product mass that are consistent with theoretical mass found by the logician ChemDraw Ultra 8.0. Diverse analyses of spectrometrical data done on compounds have been confirmed typical functions and other groups forming the sequences of each structure.

Pharmacology

The scaffold (**scheme 1**) has advantageous properties: low molecular weight, reasonable *ClogP*, good hydrogen bond donating and accepting capabilities (table 1), easy and economical synthetic routes [17,18].

Table 1: Synthetic molecules have physical properties compatible with reasonable pharmacokinetics and drug availability

Rule	**Molecular weight**	***C*log*P***	**No. of H bond donors**	**No. of H bond acceptors**	**No. of criteria met**
	< 500	**< 5**	**< 5**	**< 10**	**at least 3**
1	255	3.96	2	3	all
2	289.5	4.673	2	3	all
3	285	4.229	2	3	all
4	285	4.229	2	3	all

In this table, we noted that compounds respected all of the criteria and then they would endow to pharmaceutical activities.

The results of their biological activity were summarized in the table 2.

Table 2: IC_{50} values and anti-parasitic activity showed by the synthesized products

Compounds	IC_{50} values (µM)	Trypanocidal activity
1	>100	little
2	281.208±18.825	little
3	>100	little
4	>100	little

All products showed little or no activity on *Trypanosoma bruceibrucei* with their half inhibitory concentration (IC_{50}) values higher than 100 micromolar (100 µM). This low anti-parasitic activity has been attributed to them according to the scale of trypanocidalacitivity described in the works of Du et *al*., 2002 and Fujii et *al*., 2005 [19,20]. In their study, compounds that have IC_{50} values below 10 µM can be considered as trypanocidal molecules, when IC_{50} values are between 10 and 100 µM, they are considered as moderate antitrypanosomal. The compounds which have their IC_{50} higher than 100 µM presented little or no activity (table 2).

Conclusion

In this study, we studied N(4)-phenyl-3-thiosemicarbazones of benzaldehyde and derivatives. All products in spite of their physical properties compatible with reasonable pharmacokinetics and drug availability exhibited little activity on the trypanosome used. Other pharmaceutical properties of molecules will be explored on some microbes or parasites.

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