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Short Communication

1-*N*-Substituted Thiocarbamoyl-3-Phenyl-2-Pyrazolines: Synthesis and In Vitro Antiamoebic Activities

Mohammad Abid, Amir Azam *

Department of Chemistry, Jamia Millia Islamia, Jamia Nagar, New Delhi-110025, India

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Abstract

The title compounds were prepared by reaction of Mannich bases with various *N*-4 substituted thiosemicarbazides. The chemical structures of the compounds were proved by means of their UV, IR, ^{1}H NMR, ^{13}C NMR spectroscopic data and elemental analyses. The in vitro antiamoebic activities of these compounds were evaluated by microdilution method against *HM1:IMSS* strain of *Entamoeba histolytica* and compared with the standard drug, metronidazole. It was concluded that 3-chloro and 3-bromo substituents on the phenyl ring at position 3 of the pyrazoline ring enhanced the antiamoebic activity. Compounds **9, 17, 18, 20** and **21** showed less IC_{50} value than metronidazole.

Keywords: Pyrazoline; Mannich base; Thiocarbamoyl; Thiosemicarbazide; *Entamoeba histolytica*

1. Introduction

Parasitic protozoa continue to beleaguer and kill millions of people in the subtropical and tropical regions of the world. The amitochondrial protist *E. histolytica* is estimated to infect up to 10% of the world's population. Fifty million cases of amoebic dysentery and liver abscess are reported each year [1]. These infections result in approximately 50,000 to 100,000 deaths annually [2]. Amoebiasis is primarily treated with the drug metronidazole [1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole], even though significant side effects, such as neurological complications, and the possible selection of a resistant *E. histolytica* strain have been reported [3–6]. In addition, treatment failures among patients with amoebiasis often raise the possibility of drug resistance. Therefore it is desirable to search for new leads for amoebicidal drugs.

Pyrazoles and their reduced forms pyrazolines are well known nitrogen containing heterocyclic compounds and various procedures have been developed for their syntheses [7]. As a result, a wide variety of pyrazoles and pyrazolines have hitherto been described in the literature [7,8]. The interest of scientists in such compounds has been stimulated by their various promising pharmacological properties [9]. As evident from the literature, it was noted that very little research has been carried out on 1-*N*-substituted thiocarbamoyl-3-phenyl-2-pyrazolines [10], but no work has been done on screening of these compounds against *E. histolytica*. Earlier we have reported different heterocyclic thiosemicarbazones, their transition metal complexes and in vitro screening against *E. histolytica* [11–13] and their in vivo and cytotoxicity studies are in progress. As literature survey reveals the pharmacological importance of pyrazolines and their derivatives, this prompted us to synthesize new 1-*N*-substituted thiocarbamoyl-3-phenyl-2-pyrazoline derivatives **1-21** (Table 1) and their in vitro screening against *HM1:IMSS* strain of *E. histolytica*. To the best of our knowledge this is the first report against *E. histolytica*.

2. Chemistry

The Mannich reaction of various ketones with formaldehyde and dimethylamine hydrochloride generates the Mannich base precursor [14]. The reaction is sensitive to both the amount of hydrochloric acid and ethanol present. The reaction works best when a minimum amount of ethanol and 2 μL of acid/mmol ketone is added. The methyl phenyl ketone gave high yields above 80%, while the yields for 3-bromo

* Corresponding author. Tel.: 0091 11 26981717 / 3253, fax: 0091 11 26980229 / 0091-11-26981232.
E-mail address: sumbul@yahoo.co.in (A. Azam).

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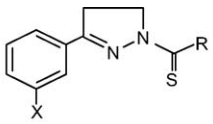
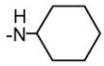
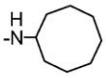
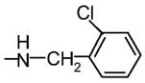
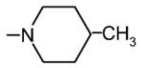
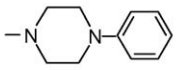
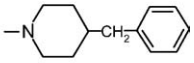
Table 1
Structure of 1-N-substituted thiocarbamoyl-3-phenyl-2-pyrazolines (**1-21**)



Compound	X	R
1.	H	
2.	Br	
3.	Cl	
4.	H	
5.	Br	
6.	Cl	
7.	H	
8.	Br	
9.	Cl	
10.	H	
11.	Br	
12.	Cl	
13.	H	
14.	Br	
15.	Cl	
16.	H	
17.	Br	
18.	Cl	
19.	H	
20.	Br	
21.	Cl	

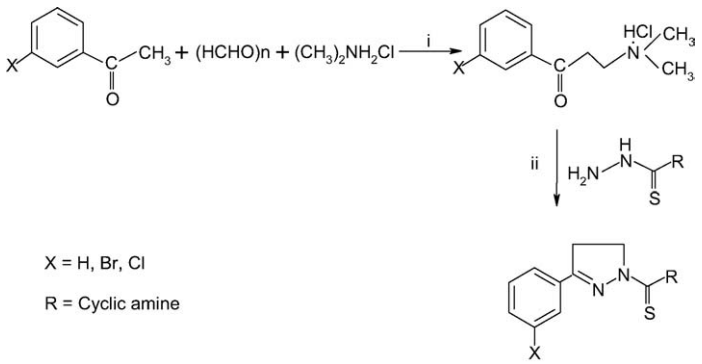
and 3-chloro acetophenones in the Mannich reaction were lower in the range of 40-60%. All the thiosemicarbazides were prepared by the method reported by O'Sullivan [15]. The condensation of Mannich reaction product with *N*-4 substituted thiosemicarbazides by different cyclic amines lead to the formation of 1-*N*-substituted thiocarbamoyl 3-phenyl-2-pyrazolines **1-21** (Scheme 1). According to the currently accepted mechanism, the formation of 1-*N*-substituted thiocarbamoyl-3-phenyl-2-pyrazolines is favored via thiosemicarbazone formation, which undergo cyclization under basic conditions to form desired pyrazoline ring in all the compounds [16,17]. All the compounds were purified by column chromatography to give crystalline solid compounds, but in low yield (9-25%). The compounds are stable in the solid as well as in the solution state. The structure of the compounds was confirmed by IR, ^{1}H NMR, ^{13}C NMR, electronic spectra and elemental analysis. The compounds are insoluble in water but soluble in methanol, ethanol and DMSO.

3. Pharmacology

All the 1-*N*-substituted pyrazoline derivatives **1-21** were screened in vitro for antiamoebic activity against *HM1:IMSS* strain of *E. histolytica* by microdilution method [18]. *E. histolytica* trophozoites were cultured in TYIS-33 growth medium as described previously [19] in wells of 96-well microtiter plate. Each compound tested was serially diluted and added to the growing trophozoites in microtiter plate. Effect on growth of trophozoites was monitored microscopically at regular interval and quantitative estimation of the drug action was made by protein estimation. The % inhibition of amoeba was calculated from the optical densities of the control and tested wells and was plotted against the logarithm of concentration of the drug tested. Linear regression analysis was used to determine the best fitting straight line from which IC_{50} value was found.

4. Results and Discussion

The synthesis of 1-*N*-substituted 3-phenyl-2-pyrazolines (**1-21**) was done by cyclization of Mannich bases with various *N*-4 substituted thiosemicarbazides in methanol under basic conditions. The product mixture contained only unreacted Mannich base and the cyclization product, which was purified by column chromatography using silica gel $60F_{254}$ eluted with dichloromethane: methanol (98:2). The yield of cyclised product in unsubstituted thiosemicarbazide was in the range of 50-70%; and 9-25% in the case of substituted thiosemicarbazides. Analytical data of the compounds are in good agreement with their composition. The structure of the compounds were established by means of their spectral data (IR, UV, ^{1}H NMR, ^{13}C NMR) and elemental analyses and are presented in Table 1.



Scheme 1. (i) Ethanol, hydrochloric acid, reflux; (ii) Methanol, NaOH, reflux.