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Short communication

Synthesis of new 2-(5-substituted-3-phenyl-2-pyrazolinyl)-1, 3-thiazolino[5,4-*b*]quinoxaline derivatives and evaluation of their antiamoebic activity

Asha Budakoti, Abdul Roouf Bhat, Amir Azam*

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

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Abstract

In an effort to develop potent antiamoebic agents, we have synthesized chalcones (**1**–**8**), amino-5-substituted-(3-phenyl(2-pyrazolinyl))methane-1-thione derivatives (**1a**–**8a**) and 2-(5-substituted-3-phenyl-2-pyrazolinyl)-1,3-thiazolino[5,4-*b*]quinoxaline derivatives (**1b**–**8b**) and evaluated for their *in vitro* antiamoebic activity against HM1:IMSS strain of *E. histolytica*. All the compounds were characterized by electronic, IR, ^{1}H NMR and mass spectroscopic data. It was observed that the antiamoebic activity enhances on modifying the structure of chalcones to the pyrazolines and further to quinoxalines. The MTT assay was performed on human kidney epithelial cell line to check the cytotoxicity of the compounds and the results were compared with metronidazole. Compound **6b** showed better antiamoebic activity and less toxicity than metronidazole.

Keywords: Chalcone; Pyrazolines; Thiocarbamoyl; Quinoxaline; Antiamoebic activity

1. Introduction

Ameobiasis is the infection of the human gastrointestinal tract by *Entamoeba histolytica* [1]. *E. histolytica* causes approximately 50 million cases and approximately 100,000 deaths annually. Amoebic liver abscesses are the most frequent and severe clinical presentations of amoebiasis. Symptomatic patients, typically present abdominal pain, tenderness, diarrhea and bloody stools. The drugs used to eradicate *E. histolytica*, such as nitroimidazoles, have been shown to pose several important problems as mutagenic and toxic for the host when they are used at high doses, amoeba strains are able to develop resistance to these drugs [2]. Amoebiasis is treated with the drug metronidazole, even though significant side effects, such as neurological complication and possible selection of a resistant *E. histolytica* strain have been reported [3]. Therefore, the development of new alternative antiamoebic drugs devoid of side effects is still needed.

Among the various classes of nitrogen containing heterocyclic compounds, quinoxaline derivatives are the important components of several pharmacologically active compounds [4–9]. Although rarely described in nature, synthetic quinoxaline ring is part of number of antibiotics such as echinomycin and actinomycin, which are known to inhibit the growth of Gram-positive bacteria and are also active against various transplantable tumors [10–12]. There are many examples of biologically active quinoxalines, which showed very interesting pharmacological properties such as antibacterial, antiviral, anticancer, antifungal, anthelmintic, and insecticidal [13–18]. All literature survey reveals the pharmacological importance of quinoxalines. The new quinoxaline derivatives, **1b**–**8b**, were synthesized and screened *in vitro* for their ability to inhibit the growth of *E. histolytica*.

* Corresponding author. Tel.: +91 11 26981717/3253; fax: +91 11 26980229/1232.
E-mail address: amir_sumbul@yahoo.co.in (A. Azam).

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2. Results and discussion

The synthetic routes of the proposed compounds **1**–**8**, **1a**–**8a** and **1b**–**8b** are outlined in Scheme 1. Claisen–Schmidt condensation between acetophenone and aromatic aldehydes in the presence of methanolic solution of sodium hydroxide resulted in the formation of chalcones (**1**–**8**) in excellent yields (49–93%). They were characterized by UV–vis, IR and ^{1}H NMR spectroscopic techniques. Cyclisation of different chalcones with thiosemicarbazide under basic condition in refluxing ethanol leads to the formation of new amino-5-substituted-(3-phenyl(2-pyrazolinyl))methane-1-thione (**1a**–**8a**) in modest yields (11–24%). They were also fully characterized using various spectroscopic techniques. The 2-(5-substituted-3-phenyl-2-pyrazolinyl)-1,3-thiazolino[5,4-*b*]quinoxaline derivatives (**1b**–**8b**) were obtained in (16–49%) yields by heating at reflux amino-5-substituted-(3-phenyl(2-pyrazolinyl))methane-1-thione (**1a**–**8a**) with 2,3-dichloroquinoxaline. The progress of each reaction was monitored by thin layer chromatography. Compounds **1b**–**8b** were characterized by IR, UV–vis, ^{1}H NMR, ^{13}C NMR and mass spectroscopy. The purity of all the compounds was checked by elemental analysis.

2.1. IR and electronic spectral studies

The positions of IR band provide significant indication for the formation of **1**–**8**, **1a**–**8a** and **1b**–**8b**. The bands due to ν(C=O) and ν(C=C) stretch at (1669–1750) cm^{-1} and (1523–1580) cm^{-1}, respectively, favors the formation of chalcone derivatives (**1**–**8**). The absence of ν(C=O) and ν(C=C) bands in the IR spectra of **1a**–**8a** obtained by cyclisation of chalcone **1**–**8** shows the formation of **1a**–**8a**. Compounds **1a**–**8a** showed intense bands in the region 1333–1370 cm^{-1} due to the ν(C=S) stretch of the thiocarbamoyl group. The IR spectra of all the compounds showed ν(C=N) stretch at 1542–1590 cm^{-1} due to the ring closure. In addition, the absorption bands at 1024–1122 cm^{-1} were attributed to the ν(C–N) stretch vibrations, which also confirm the formation of desired pyrazoline ring in all the compounds. The compounds showed sharp bands in the region 3228–3454 cm^{-1} due to the ν(NH) stretch. Compounds **1a**–**8a** were further reacted with 2,3-dichloroquinoxaline to get compounds **1b**–**8b**. The bands due to ν(NH) stretch and ν(C=S) were absent in these compounds. Selected diagnostic bands of the IR spectra of 2-(5-substituted-3-phenyl-2-pyrazolinyl)-1,3-thiazolino[5,4-*b*]quinoxaline derivatives (**1b**–**8b**) showed intense bands at 839–945 cm^{-1} due to the ν(C–S) stretch. The compounds show two strong bands at 1486–1563 and 1517–1510 cm^{-1} due to ν(C=N) stretch of azomethine nitrogen of pyrazoline ring and quinoxaline ring, respectively. In addition, the absorption band at 1078–1192 cm^{-1} attributed to the ν(C–N) stretch vibrations, which also confirm the formation of desired pyrazoline ring in all the compounds [19].

The electronic spectra of the cyclised pyrazoline analogues studied in the UV region in methanol, exhibited three absorption bands at 371–290, 270–236 and 223–205 nm assignable to n → π*, π → π* and n → σ* transitions, respectively. The band at 371–292 nm was assigned to the transition involving the thione portion (C=S) of thiocarbamoyl group. The two other absorption bands at 288–236 and 232–205 nm were due to π → π* transition of phenyl ring and n → σ* transition of azomethine nitrogen, respectively. The UV spectral data of **1b**–**8b** were also studied which showed the same type of transitions as observed in compounds **1a**–**8a**. It showed three spectral bands at 388.3–299, 287–248 and 239–204 nm assigned to n → π*, π → π* and n → σ* transitions of thiocarbamoyl group (C–S–C), aromatic ring and azomethine nitrogen, respectively.

2.2. Nuclear magnetic resonance spectral studies

Further evidence for the formation of **1**–**8**, **1a**–**8a** and **1b**–**8b** was obtained by ^{1}H NMR spectroscopy, which provide diagnostic tools for the positional elucidation of the protons. Assignment of the signals are based on the chemical shifts and intensity patterns. Two doublets in the ^{1}H NMR spectra of chalcones (**1**–**8**) in the region (6.86–7.74) and (5.82–7.66) ppm appears due to (–CO–CH=) and (=CH–Ar) protons favor their formation. In the ^{1}H NMR spectra of the cyclised product of chalcone, **1a**–**8a**, pyrazoline protons H_A and H_B are geminal protons at C_4 carbon, appears in the region 3.90–3.09 and 3.32–3.98 ppm as doublet of doublets in all compounds. The CH proton also appeared as doublet of doublets in the region of 6.32–5.40 ppm due to vicinal coupling with two non-equivalent geminal protons of C_4 carbon. These protons H_A and H_B protons at C_4 carbon were slightly shifted in case of quinoxaline compounds **1b**–**8b** and appears in the region 3.99–3.11 and 3.80–2.78 ppm as doublet of doublets in all quinoxaline compounds. The CH proton also appeared as doublet of doublets in the region of 6.58–5.11 ppm due to vicinal coupling with two non-equivalent geminal protons of C_4 carbon. The NH proton of different substituted thiocarbamoyl pyrazoline compounds showed a doublet at 9.24–7.17 ppm. Compounds **1a**–**8a** and **1b**–**8b** were additionally characterized by ^{13}C NMR spectroscopy. In the ^{13}C NMR spectra, the C_4 and C_5 carbons of the pyrazoline ring in compounds **1a**–**8a** resonate at 37.43–37.06 and 62.98–60.49 ppm, respectively. The phenyl–C resonates at 149.93–132.71 ppm. All the pyrazoline compounds showed a signal at 189.23–170.16 ppm, which was assigned to azomethine carbon of pyrazoline ring. Thiocarbamoyl carbon (C=S) displayed a signal at 169.42–204.23 ppm. The quinoxaline compounds **1b**–**8b** showed two signals at 151.66–152.36 and 140.11–143.35 ppm due to azomethine carbon of the pyrazoline ring and quinoxaline ring, respectively. Thiocarbamoyl carbon (C–S–C) displayed a signal at 143.07–139.31 ppm in all the compounds.

2.3. ESI-MS analysis

The characteristic peaks observed in the mass spectra of quinoxaline derivative (**8b**) are summarized in Scheme 2. The mass spectrum exhibits molecular ion peaks and contains fragments that confirm the quinoxaline structure in all the compounds.