

Synthesis of some pyrazolone derivatives from ciprofloxacin and study of their cytotoxicity

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Article Info

Received: 31 March 2010

Accepted: 9 May 2010

Available Online: 30 May 2010

DOI: 10.3329/bjp.v5i1.4693

Cite this article:

Devnath HP, Islam MR. Synthesis of some pyrazolone derivatives from ciprofloxacin and study of their cytotoxicity. Bangladesh J Pharmacol. 2010; 5: 30-33.

Abstract

Three pyrazolone derivatives with pyrazole ring extension were synthesized from ciprofloxacin by treating the parent compound with hydrazine derivatives. All the products have been characterized by IR and ¹H-NMR spectral analysis. These derivatives showed potential cytotoxicity against brine shrimp nauplii than the ciprofloxacin.

Introduction

Ciprofloxacin is a broad-spectrum fluoroquinolone antibiotic used in the treatment of a wide range of mild to moderate Gram-positive and Gram-negative infections. As ciprofloxacin, has a keto and a carboxylic acid group in the ortho position, an extra pyrazole ring system on the ciprofloxacin molecule can be created. Pyrazolone and its derivatives are used for their analgesic (Gursoy et al., 2000), anti-inflammatory (Satyanarayana and Rao, 1995), antipyretic (Manna et al., 1992), antiarrhythmic, antifungal (Bekhit and Fahmy, 2003), muscle relaxing, psychoanaleptic, anticonvulsant, enzyme inhibiting (Regan et al., 2003), antidiabetic and antibacterial activities. So, the chemistry of pyrazolone and its derivatives is particularly interesting because of their potential application in medicinal chemistry. In continuation of our work to investigate the cytotoxicity (Islam et al., 2001) of pyrazolone derivatives, three pyrazolone derivatives from ciprofloxacin have been synthesized and we report herein the result of cytotoxicity.

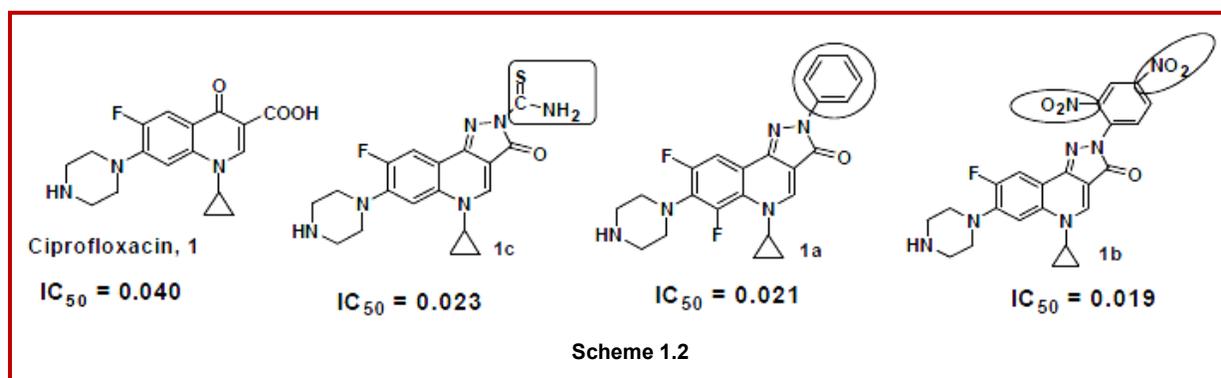
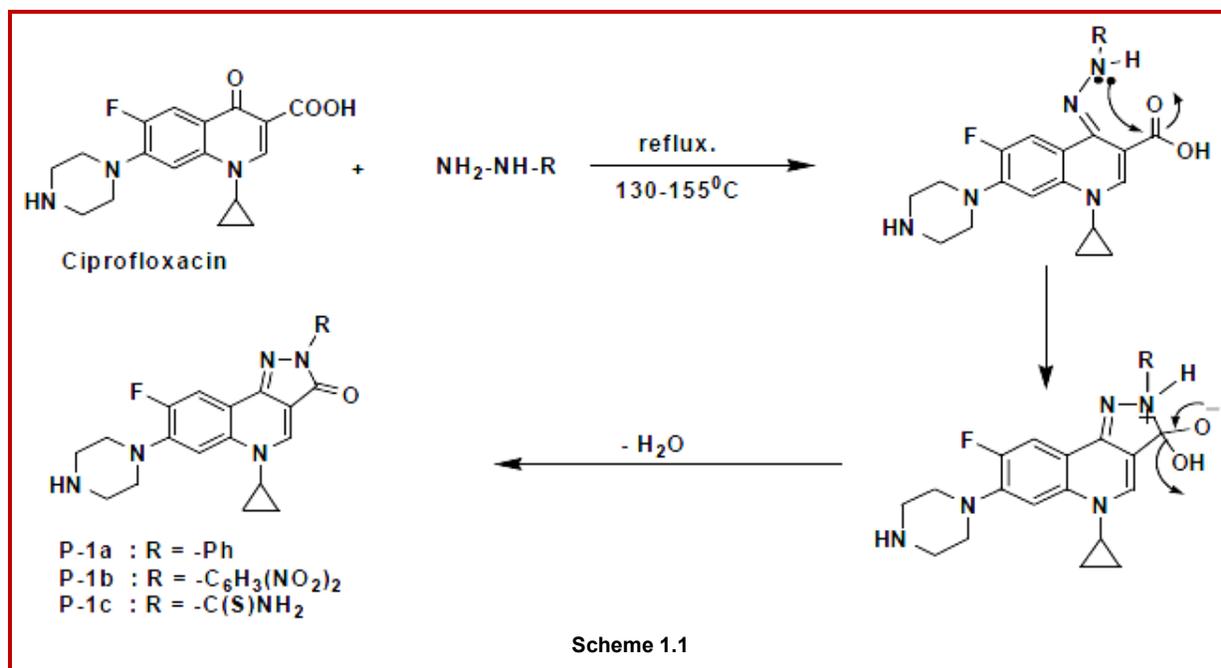
Materials and Methods

All melting points were recorded by thin disk method

on a "Fischer Johns" electro thermal melting point apparatus. Infrared spectra were recorded on DR-8001, Shimadzu FTIR spectrophotometer as a solid, which was finely grounded in a small agate mortar with a drop of nujol (liquid hydrocarbon) as a mull and also in KBr disk. ¹H-NMR spectra were measured by WP 400-NMR spectrometer, deuterated solvents such as dimethylsulfoxide (DMSO-d₆) were used as solvents and the chemical shifts were quoted as δ-value relative to tetramethyl silane (TMS, δ=0) as an internal standard. The purity of compounds was checked by TLC on silica gel plates and iodine was used as a visualizing agent.

The compound, 5-Cyclopropyl-8-fluoro-2-phenyl-7-piperazin-1-yl-2,5-dihydro-pyrazolo [4,3-c]quinolin-3-one, **1a** (scheme 1.1) was prepared by the mixture of ciprofloxacin (1.0238 g, 2.7229 mmol) in 10 mL methanol and an equivalent amount of phenyl hydrazine (0.2941 g, 2.7229 mmol) taken in 50 mL two necked quick fit round bottom flask, was heated in an oil bath at 120-130°C for 2.5 hours. The color of the reaction mixture was changed to white brown during the reflux period. The reaction mixture was allowed to cool at room temperature and was kept overnight to be





precipitated out. Then the reaction mixture was treated with water, immediate pale yellow solid came out. The crude product was purified by recrystallization from ethanol and identified as 5-cyclopropyl-8-fluoro-2-phenyl-7-piperazin-1-yl-2,5-dihydro-pyrazolo[4,3-c]quinolin-3-one, **1a**. The compound 5-cyclopropyl-2-(2,4-dinitro-phenyl)-8-fluoro-7-piperazin-1-yl-2,5-dihydro-pyrazolo[4,3-c]quinolin-3-one, **1b** (scheme 1.1) was synthesized by the refluxing ciprofloxacin with phenyl hydrazine according to above process. The compound 5-cyclopropyl-8-fluoro-3-oxo-7-piperazin-1-yl-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-carbothioic acid amide, **1c** was synthesized by the refluxing ciprofloxacin with thiosemicarbazide according to above process (scheme 1.1).

Hatching and maintenance of brine shrimp (Islam and Mohsin, 2007; Solis et al, 1993): Brine shrimps (*Artemia salina*) were used as test animal for the investigation of cytotoxicity. The essential condition for brine shrimp (temperature 27-30°C, salinity 30-35 ppt, pH 7.5-8.5, and

strong aeration) was established by mixing sodium chloride salts in water. Natural or artificial light (at night) was required and constant oxygen supply was carried out by bubble pump machine. After obtaining the desired condition, about one teaspoon of brine shrimp eggs was added to the beaker. After 12 hours hatching aggregated brine shrimp nauplii were collected in another beaker and rinse with fresh water and applied for testing.

Preparation of test sample: 1.6 mg of each compound in a sample vial was weighed by an analytical balance. Then 1.6 mL of DMSO were added to each vial and vigorously shaken to prepare stock solution. 5 mL of sea water was given in each test tube. With the help of micropipette specific volumes of samples were transferred from the stock solution to the test tube to get the final sample concentration of 0.05, 0.1, 0.15, and 1.0 µg/mL. Then 10-15 brine shrimps, immediately germinated from brine shrimp eggs, were placed in each test tube.

Table I

Physical data of the synthesized compounds				
Compounds	% of yield	Melting points (°C)	Rf values (CH ₂ ClMeOH =9:1)	Color of compounds
1a	88	215	0.74	White
1b	82	203	0.73	Yellow
1c	88	220	0.69	White brown

Table II

Cytotoxicity activity (IC ₅₀ , after 4 hours) of synthesized compounds against brine shrimps				
Tested compounds	Concentration of sample (µg/mL)	% of mortality	IC ₅₀ (in µg/mL)	Remarks
1	0.1	63.6	0.04	Moderately active
	0.1	81.8		
	0.2	80.0		
	1.0	80.0		
1a	0.1	71.4	0.02	Highly active
	0.1	76.7		
	0.2	78.9		
	1.0	80.0		
1b	0.1	81.8	0.02	Very highly active
	0.1	81.8		
	0.2	90.0		
	1.0	100.0		
1c	0.1	80.0	0.02	Highly active
	0.1	88.9		
	0.2	88.9		
	1.0	100.0		

IC₅₀: 0.01-0.02, very highly active; IC₅₀: 0.02-0.03, highly active; IC₅₀: 0.03-0.04 moderately active; IC₅₀: 0.04-0.05, Active; IC₅₀: >45, weakly active

Counting of nauplii: After 1, 2, 3 and 4 hours, the test tubes were observed and the number of survived nauplii in each test tube was counted and results were noted. The percentage of mortality of brine shrimp was calculated for each sample that gives different mortality for different concentrations. An approximate linear correlation was observed when logarithm of concentration was plotted against percentage of mortality and the values of IC₅₀ were calculated for each sample. The IC₅₀ represents the concentration of a compound, which will kill, or inactive 50 percent of the test animal. IC₅₀ is inversely proportional to the toxicity of a compound, i.e. the lower the IC₅₀ the higher the toxicity.

Results

Physical constants give the preliminary idea about the formation of new compounds (Table I). The resulting

spectral data analyses of synthesized compounds:

Compound 1a: IR (ν_{\max} , KBr, cm⁻¹): 3200.6 cm⁻¹(s, sh, VN-H); 3070 cm⁻¹(s, sh, VC-H, aromatic); 920 cm⁻¹(s, sh, VC-H, aromatic, out of plane); 2961 cm⁻¹(w, sh, VC-H CH₂); 1447.5 and 1378 cm⁻¹(s, sh, VC-H bending, CH₂); 1612 and 1490 cm⁻¹(s, sh, VC-C, aromatic); 1604 cm⁻¹(s, sh, VC-N); 1700 cm⁻¹(s, sh, VC=O, lactam), 1576 cm⁻¹(s, sh, VN-H, bending); 1652 cm⁻¹(m, VC=C, alkene); 1028 cm⁻¹(s, sh, VC-N amine); 858 cm⁻¹(s, br., VN-H, wagging); 752 cm⁻¹(s, sh, VC-F). ¹HNMR (400 MHz, DMSO-d₆): 8.0866 (m, 4H, -CH₂, cyclopropane); 1.523 (t, 1H, -CH); 2.53 (m, 4H, -cH₂); 3.48 (d, 4H, -cH₂); 15.251 (s, 1H, -NH); 4.562 (d, 1H, =CH); 7.58 (m, 5H, aromatic); 8.407 (d, 1H, aromatic); 8.67 (s, 1H aromatic).

Compound 1b: IR (ν_{\max} , KBr, cm⁻¹): 3296 cm⁻¹(s, sh, VN-H); 3025 cm⁻¹(s, sh, VC-H, aromatic); 825.5 cm⁻¹(s, sh, VC-H, aromatic, out of plane); 2856 cm⁻¹(w, sh, VC-H, CH₂); 1447.5 and 1378 cm⁻¹(s, sh, VC-H bending, CH₂); 1549.7 and 1358.8 cm⁻¹(m, VN=O, Nitro); 1612 and 1490 cm⁻¹(s,

sh, $\nu_{C=C}$, aromatic); 1723 cm^{-1} (s, sh, $\nu_{C=N}$); 1627 cm^{-1} (s, sh, $\nu_{C=O}$, lactam), 1576 cm^{-1} (s, sh, ν_{N-H} , bending); 1652 cm^{-1} (m, $\nu_{C=C}$, alkene); 1028 cm^{-1} (s, sh, ν_{C-N} amine); 825 cm^{-1} (s, br., ν_{N-H} , wagging); 744 cm^{-1} (s, sh, ν_{C-F}). $^1\text{H-NMR}$ (400 MHz, DMSO- d_6): 8 0.856 (m, cyclopropane); 1.234 (t, 1H, -CH); 2.50 (m, 4H, -CH₂); 3.430 (t, 4H, -CH₂); 15.211 (s, 1H, -NH); 4.563 (d, 1H, =CH); 7.550 (dd, Hb); 7.959 (d, Ha); 7.268 (d, Hc); 8.672 (s, Hd aromatic); 8.325 (d, He aromatic).

Compound 1c: IR (ν_{max} , KBr, cm^{-1}): 3373 cm^{-1} (s, sh, ν_{N-H}); 3184 cm^{-1} (s, sh, ν_{C-H} , aromatic); 824.5 cm^{-1} (s, sh, ν_{C-H} , aromatic, out of plane); 2972 cm^{-1} (w, sh, ν_{C-H} , CH₂); 1452 and 1374 cm^{-1} (s, sh, ν_{C-H} bending, CH₂); 1576 and 1490 cm^{-1} (s, sh, $\nu_{C=C}$, aromatic); 1683 cm^{-1} (s, sh, $\nu_{C=N}$); 1616 cm^{-1} (s, sh, $\nu_{C=O}$, lactam), 1576 cm^{-1} (s, sh, ν_{N-H} , bending); 1653 cm^{-1} (m, $\nu_{C=C}$, alkene); 1024 cm^{-1} (s, sh, ν_{C-N} amine); 1292 cm^{-1} (s, sh, $\nu_{C=S}$), 825 cm^{-1} (s, br., ν_{N-H} , wagging); 730 cm^{-1} (s, sh, ν_{C-F}); $^1\text{H-NMR}$ (400 MHz, DMSO- d_6): 8 0.858 (m, 4H, -CH₂, cyclopropane); 1.432 (t, 1H, -CH); 2.55 (m, 4H, -CH₂); 3.45 (t, 4H, -CH₂); 15.205 (s, 1H, -NH); 4.56 (d, 1H, =CH); 4.305 (s, 2H, -NH₂); 8.502 (d, 1H, aromatic); 8.60 (s, 1H aromatic).

Discussion

In the present work, the synthesized compounds were investigated for their property as biologically active agents by brine shrimp lethality bioassay. Among them the compounds **1b** are very highly active. **1a** and **1c** are highly active but ciprofloxacin is moderately active.

The chemical structure of a drug is important as the relatively minor modification in the drug molecule may result a major change in pharmacological properties.

The synthesized compounds **1a**, **1b** and **1c** show better bio-activity property which provide that pyrazolone derivatives of ciprofloxacin may be very important in producing anti-cancer activity. The substituted benzene ring by -NO₂ group of the 5-cytopropyl-2-(2,4-dinitrophenyl)-8-fluoro-7-pperezin-1-yl-2,5-dihydro-pyrazolo [4,3-c] quinolin-3-one, **1b** show better activity than unsubstituted benzene. The increasing order of the activity from our research work of the synthesized compounds is $1 < 1c < 1a < 1b$ (Scheme 1.2).

Acknowledgement

The authors express their sincere thanks to Md. Lokman Hossain, Okayoma University, Japan for supplying the $^1\text{H-NMR}$ spectra of the synthesized compounds.

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