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ORIGINAL ARTICLE

Synthesis, characterization and *in vitro* antimicrobial activity of some novel 5-substituted Schiff and Mannich base of isatin derivatives

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Abstract With the aim of developing potential antimicrobials, a series of novel Ciprofloxacin methylene isatin derivatives incorporating different aromatic aldehydes were synthesized and characterized by FTIR, ^{1}H NMR, Mass spectroscopy and bases of elemental analysis. In addition, the *in vitro* antibacterial and antifungal properties were tested against some human pathogenic microorganisms by employing the disc diffusion technique. A majority of compounds were showing activity against several of the microorganisms. The relationship between the functional group variation and the biological activity of the evaluated compounds is discussed. From comparisons of the compounds, **3c** was determined to be the most active compound.

1. Introduction

Microbial infections are a growing problem in contemporary medicine, and the use of antibiotics is common across the world. Antimicrobials are among the most commonly purchased drugs worldwide. They are essential treatments especially in the developing world where infectious diseases are a common cause of death. In the past 20 years, the incidence of microbial infection has increased on alarming levels over the world as a result of antimicrobial resistance. An increasing number of immuno-compromised patients are a result of HIV infection, cancer chemotherapy and organ transplantation which are the major factors contributing to this increase. The health problem demands to explore and synthesize a novel class of antimicrobial compounds effective against pathogenic microorganisms that developed resistance to the antibiotics used in the current regimen (Yu and Huiyuan, 2002; Koca et al., 2005).

Isatin (1H-indole-2, 3-dione), an endogenous compound identified in many organisms, shows a wide range of biological

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activities (Pandeya et al., 2005). The isatin ring is a prominent structural motif found in several pharmaceutically active compounds. This is mainly due to the easy synthesis and the importance of pharmacological activity. Therefore, the synthesis and selective functionalization of isatins have been the focus of active research area over the years (Lian-Shun et al., 2011). Isatin derivatives are reported to show antibacterial (Praveen et al., 2011) and antifungal (Amalraj et al., 2003) activities.

Fluoroquinolones on the other hand have emerged as one of the dominant classes of chemotherapeutic drugs for the treatment of various bacterial infections in both community and hospital settings (Emmerson and Jones, 2003). These antibacterial agents act by inhibiting DNA gyrase (the principal target in gram-negative bacteria) and topoisomerase IV (the primary target in gram-positive bacteria) (Charifson et al., 2008). Other class of antibiotic, such as anthracyclines (Miller and Stoodley, 2011) is used in chemotherapy regimes, and is topoisomerase II inhibitors by way of drug-DNA intercalation.

Sriram et al. reported Gatifloxacin and Ciprofloxacin methylene isatin derivatives, and a few compounds were more potent than the parent drugs against some of the bacteria (Sriram et al., 2005, 2006). Recently Lian-Shun (Feng et al., 2010) reported a series Balofloxacin ethylene isatin derivatives and 8-OCH_3 Ciprofloxacin methylene and ethylene isatin derivatives and showed their *in vitro* activity against some mycobacteria (Lian-Shun et al., 2010, 2011). Further, different 5-substituted isatin derivatives were also shown to possess a wide range of antimicrobial properties (Kandile et al., 2011).

In view of the facts mentioned above and as part of our initial efforts to discover potentially active new agents, 12 novel Ciprofloxacin methylene isatin derivatives containing different aromatic aldehydes (**3a–3l**) were synthesized and evaluated for their antimicrobial activity.

2. Materials and methods

2.1. General

All solvents used were of laboratory grade and were obtained from SD fine chemicals (Mumbai, India), and Merck (Mumbai, India). Ciprofloxacin and Ketoconazole are received as gift samples from Dr. Reddys laboratories, Hyderabad, India. Melting points were determined in open glass capillary tubes and are uncorrected. Compounds were routinely checked for their purity on Silica gel G (Merck) Thin layer chromatography (TLC) plates. Iodine chamber and UV lamp were used for visualization of TLC spots. The IR spectra were recorded in KBr pellets on (BIO-RAD FTS) FT-IR spectrophotometer. ^{1}H NMR spectra were recorded on Bruker DPX-300 NMR spectrometer in DMSO-d_6 using tetramethylsilane (TMS) as an internal standard. The chemical shifts are reported in ppm scale. Elemental data for C, H, and N were within $\pm 0.4\%$ of the theoretical values.

2.2. Test microorganisms and medium

All the microorganisms used in this study were purchased from CL laboratories, Chennai, India. All the synthesized compounds were screened for antimicrobial activity by paper disc diffusion technique. The antibacterial activity of the compounds was evaluated against four Gram-positive bacteria *Staphylococcus aureus* ATCC 9144, *Staphylococcus epidermidis* ATCC 155, *Micrococcus luteus* ATCC 4698 and *Bacillus cereus* ATCC 11778 and three Gram-negative bacteria *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 2853, and *Klebsiella pneumoniae* ATCC 11298. The antifungal activities of the synthesized compounds were evaluated against two fungi *Aspergillus niger* ATCC 9029 and *Aspergillus fumigatus* ATCC 46645. Bacterial strains were cultured over night at 37 °C in Mueller–Hinton broth and the yeast was cultured overnight at 30 °C in YEPDE agar for antibacterial and antifungal activity tests. Test strains were suspended in nutrient agar to give a final density of 5×10^{-5} cfu/ml.

2.3. Chemistry

2.3.1. Synthesis of 3-(4-aminophenylimino)-5-fluoroindolin-2-one (1)

The equimolar quantity of 5-fluoro isatin (0.01 mol) and 4-amino aniline (*p*-phenylene diamine) (0.01 mol) was dissolved in 50 ml of warm ethanol. To this, a few drops of glacial acetic acid were added. Then the mixture was heated to reflux for 1 h. After standing for approximately 24 h at room temperature, the products were separated by filtration, vacuum dried and recrystallized from ethanol. The compound **1** thus obtained was used in the next step without further purification. Dark brown powder, yield: 75% m.p. 242 °C. **IR:** (KBr, cm^{-1}) 3516 (NH_2), 1625 (C═N), 1682 (C═O). **^{1}H NMR** (300 MHz, DMSO-d_6, ppm, δ): 8.0 (1H, NH), 6.50–7.76 (7H, Ar—CH), 4.12 (2H, NH_2), ESI-MS: MS: *m/z* 255 (M^+). **Anal. Cald** for $C_{14}H_{10}FN_3O$: C, 65.88; H, 3.95; N, 16.46. Found: C, 65.90; H, 4.15; N, 16.66.

2.3.2. Synthesis of 7-(4-((3-(4-aminophenylimino)-5-fluoro-2-oxoindolin-1-yl)methyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (2)

To a solution of 1-cyclopropyl-6-fluoro-1,4-dihydro-7-piperazin-1-yl-4-oxo quinoline-3-carboxylic acid (Ciprofloxacin, 0.002 mol) in ethanol (50 ml), 3-(4-aminophenylimino)-5-fluoroindolin-2-one **(1)** (0.002 mol) and 37% formalin (1 ml) were added. The reaction mixture was heated under reflux for 24 h. On cooling, the precipitate was collected, washed with cold ethanol, and recrystallized from a mixture of DMF and water, to give the compound **2.** Dark brown powder, yield: 68% m.p. 296 °C. **IR:** (KBr, cm^{-1}) 3516 (NH_2), 2784 (COOH), 1625 (C═N), 1725 (C═O). **^{1}H NMR** (DMSO-d_6, 300 MHz) δ ppm: 7.12–8.12 (10H, Ar—CH), 4.46 (2H, s, N—CH_2—N of linker), 3.90 (2H, s, NH_2), 2.90 (4H, s, piperazine-4H), 3.32 (4H, s, piperazine-4H), 0.96 (2H, m, cyclopropyl-H), 1.02–1.10 (2H, m, cyclopropyl-H), 4.02 (1H, m, cyclopropyl-H), 11.52 (1H, COOH). **ESI-MS:** MS: *m/z* 598 (M^+). **Anal. Cald** for $C_{32}H_{28}F_2N_6O_4$: C, 64.21; H, 4.71; N, 14.04. Found: C, 64.45; H, 4.28; N, 14.41.

2.3.3. General procedure for the synthesis of (3a–3l)

Title compounds **(3a–3l)** were synthesized by adding 7-(4-((3-(4-aminophenylimino)-5-fluoro-2-oxoindolin-1-yl)methyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **(2)** (0.01 mol) as fraction portions with the well stirred mixture of different aromatic aldehydes (0.01 mol) in ethanol (50 ml) and few ml of glacial acetic acid. Then this mixture is refluxed for 8 h and kept aside. The product that separated out was filtered, dried and recrystallized from ethanol. The method used for the preparation and isolation of the