Synthesis and Antiviral Studies of Novel N-Sulphonamidomethyl piperazinyl Fluoroquinolones

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Selvam, et al.: Antiviral activities of fluoroquinolone

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A series of novel N-Sulphonamidomethyl piperazinyl fluoroquinolones were synthesized and screened antiviral activity. Eight compounds were synthesized through modifying the N\(^4\)-hydrogen of piperazine in fluoroquinolones with formaldehyde and sulphanomides by Mannich reactions. The structures of the synthesized compounds were characterized by means of their IR and \(^1\)H-NMR spectral data. Synthesized compounds were screened for antiviral activity against influenza A (H1N1, H3N2, H5N1) and influenza B viruses in MDCK cell culture. The antiHIV activities of the new compounds were screened for antiviral activity against replication of HIV-1(III\(_c\)) in MT-4 cells. Cytotoxicity of the synthesized compounds was also tested in mock-infected MDCK and MT-4 cells. Compound CF-SD and CF-SDM inhibits the influenza A (H1N1) and compound GF-SDM inhibit the replication of influenza A (H5N1) and B in MDCK cells. All compounds displayed cytostatic property in MT-4 cells. Among the compounds tested, GF-SDM (CC\(_{50}\)=39.44 µM) most toxic compound in this series.

Key words: Fluoroquinolone, Mannich reaction, antiHIV activity, Influenza, H5N1

Quinolone is a versatile lead molecule for designing potential bioactive agents and its derivatives were reported to possess board spectrum antimicrobial activity. A new fluoroquinolone K12, bearing o-methoxyphenylpiperazinyl group and a difluoromethoxy group at positions 7 and 8, respectively, was reported to have strong and selective antiHIV-1 activity\([1]\). The antiviral activity seemed to be related to an inhibitory effect at the RNA transcriptional level. Two K12 analogues bearing a phenyl dehydropiperidinyl moiety at position 7 were effective at inhibiting HIV-1 long terminal repeat (LTR)-driven gene expression, as well as suppressing tumor necrosis factor alpha (TNF-\(\alpha\)) and interleukin 6 (IL-6) production in blood mononuclear cells, suggesting a mechanism of action mediated by inhibition of Tat functions\([2]\). Recently, newer synthesized aryl piperazinyl fluoroquinolones were studied for anti-HIV activity\([3-6]\) and some of these compounds showed profound activity. A large number of fluoroquinolone have been synthesized and studied for wide range of antiviral activity\([7]\), but its activity against influenza virus has not been well explored. Based on these findings, some fluoroquinolones were synthesized and screened for antiviral activity against both HIV and influenza viruses.

Melting points were determined in open capillary tubes on a Thomas-Hoover melting point apparatus and are uncorrected. IR spectra were recorded using KBr pellets on a Jasco-410 infrared spectrophotometer. \(^1\)H-NMR spectra were determined on Bruker AMX 400 MH\(_z\) with tetramethylsilane as an internal standard. The sample was dissolved in DMSO-d\(_6\) and the value was measured in \(\delta\) ppm.

Synthesis of N-sulphonamidomethyl fluoroquinolones derivatives was achieved by stirring on equimolar (0.01 mol) mixture of sulphanamides (sulphadiazine and sulphadimidine), formaldehyde (37% v/v, ml) and fluoroquinolone (norfloxacin NF, ciprofloxacin CF and gatifloxacin GF) with ethanol using a magnetic stirrer for 3 h (Scheme 1). The mixture was allowed to cool over night in a refrigerator. The solid thus obtained was recrystallized from DMF with ethanol.

NF-SA yield: 65.24%, mp: 268\(^0\); IR (KBr) cm\(^{-1}\): 3349 (NH), 2365 (C-Alkyl), 1763 (C=O), 1622 (C=N), 1440 (SO\(_2\)), 670 (C-F); \(^1\)H-NMR (DMSO-d\(_6\)) \(\delta\) ppm:1.13 (t, 3H, CH\(_3\)), 2.0 (s, 2H, NH\(_2\)), 2.6 (s, 4H, -piperazinyl), 3.1 (q, 2H, -CH\(_2\)), 3.5 (s, 4H,-piperazinyl), 4.0 (s, 1H, NH), 4.13 (s, 2H,-N-CH\(_2\)-N-), 6-7.7 (m,6H, Ar-H), 11.0 (s, 1H, COOH); EI-MS (m/z):503. NF-SD yield:75.52%, mp:190\(^0\); IR (KBr)
cm⁻¹: 3352 (NH), 2365 (C-Alkyl), 1747 (C=O), 1622 (C=N), 1440 (SO₂), 673 (C-F); ¹H-NMR (DMSO-d₆) δ ppm: 1.13 (t, 3H, CH₃), 2.6 (s, 4H,-piperazinyl), 3.10 (q, 2H,-CH₂), 3.5 (s, 4H,-piperazinyl), 4.0 (s, 1H, NH), 4.13 (s, 2H,-N-CH₂-N-), 6.7-7.7 (m, 7H, Ar-H), 8.4 (s, 2H, pyrimidinyl), 11.0 (s, 1H, COOH); EI-MS (m/z): 581.

The inhibitory effects of the compounds on influenza A(H1N1,H2N2, H5N1) and B viruses replication were determined by cytopathic effect inhibition (CPE) assays in MDCK cell monolayers conducted in 96-well microplates[^8][^9]. Compounds in half-log₁₀ dilution increments were applied to cells 5–10 min before adding virus, using three wells for infection and two wells for toxicity controls. Fifty cell culture infectious virus doses (50 CCID₅₀) of virus were then added, and the plates were incubated for 3 d when inhibitor-free cell cultures were completely destroyed by virus. At this time, the mean percentage of cell viability in each set of three infected wells or set of two toxicity control wells was quantified by a neutral red dye uptake method, using 0.011% final concentration of the dye for 2 h. An Excel spreadsheet was developed for converting optical density readings to percentages of untreated control values. Concentrations of compounds reducing viral CPE by 50% (EC₅₀ values) were calculated by plotting concentration versus percent inhibition on a semi-log₁₀ graph paper. Antiviral activity and cytotoxicity of standard ribavirin were also performed by a similar method.

Compounds were tested for their inhibitory effects against replication of HIV-1 (IIIb) in MT-4 cells[^10][^11]. The MT-4 cells were grown and maintained in RPMI 1640 DM medium supplemented with 10% (v/v) heat-inactivated fetal calf serum (FCS), 2 mM-glutamine, 0.1% sodium bicarbonate and 20 µg/ml gentamicin (culture medium). Inhibitory effect of test compounds on HIV-1 replications was monitored by inhibition of virus-induced cytopathic effect in MT-4 cells and was estimated by MTT assay. Briefly, 50 µl of HIV-1 (100-300 CCID₅₀) were added to a flat-bottomed microtiter tray with 50 µl of medium containing various concentrations of compounds. MT-4 cells were added at a final concentration of 6×10⁵ cells/ml. After 5 d of incubation at 37°C, the number of viable cells were determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) method. Cytotoxicity of test compounds against mock-infected MT-4 cells was also assessed by the MTT method. AntiHIV activity and cytotoxicity of standard AZT were also performed by a similar method in MT-4 cells.
piperazine moiety of fluoroquinolone, which represents a site amenable to significant modification. We report a study of replacing the N₄-hydrogen of piperazine in fluoroquinolone with different substitutions of sulphonamide moiety via manich reactions to form N-sulphonamido- methyl fluoroquinolone derivatives.

Compound CF-SD and CF-SDM inhibited the replication of influenza A (H1N1). Their inhibitory concentration (EC₅₀) was 16 and 18 µg/ml, respectively, whereas the cytotoxic concentration (CC₅₀) was found to be more than 100 µg/ml (Table 1). The compound GF-SDM inhibited the avian flu (H5N1) with the EC₅₀ value of 19 µg/ml and CC₅₀ value of >100 µg/ml. These compound also inhibited significant activity against influenza B with an EC₅₀ value of 11 µg/ml and CC₅₀ >100 µg/ml. Other compounds investigated exhibited mild inhibitory activity against influenza viruses. All the compounds exhibited more than 100 µg/ml in uninfected MDCK cells. The standard ribavirin inhibits the replication of influenza A and B viruses in the concentration of 3.7-5.9 µg/ml and their cytotoxic concentration was found to be >100 µg/ml.

The synthesized compounds were evaluated for antiHIV activity against HIV-1 replication in acutely infected MT-4 cells (Table 2). The 50% effective concentration (EC₅₀) values of the compounds against the replication of HIV-1 were higher than the cytotoxic concentrations (CC₅₀). Whereas the standard AZT (EC₅₀) had 0.0064 µM against HIV-1 in MT-4 cells. Cytotoxic concentrations of test compounds were found to be 39-75 µM, whereas the standard AZT showed 65.9 µM in mock infected MT-4 cells. Presence of 2-pyrimidinyl/4,6-dimethyl-2-pyrimidinyl group in sulphonamide (SO₂NH₂) of N-sulphonamidomethyl piperizinyl fluoroquinolone lead molecule is essential for antiviral activity against influenza viruses. Free SO₂NH₂ of N-sulphonamidomethyl piperizinyl fluoroquinolone substitution will reduce the antiviral activity (Table 1). These lead molecules are suitable for designing newer derivatives against influenza viruses based upon promising antiviral activity seen.

ACKNOWLEDGEMENTS

Author is grateful to Dept of Science and Technology for award of BOYSCAST fellowship for this research work.

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Antibacterial Activity of Ethanol Extract of Andrographis paniculata

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In the present study the ethanol extract of the aerial part of Andrographis paniculata was prepared and evaluated for antimicrobial activity against eleven bacterial strains by determining minimum inhibitory concentration and zone of inhibition. Minimum inhibitory concentration values were compared with control and zone of inhibition values were compared with standard ciprofloxacin in concentration 100 and 200 µg/ml. The results revealed that, the ethanol extract is potent in inhibiting bacterial growth of both Gram-negative and Gram positive bacteria.

Key Words: Antibacterial activity, Andrographis paniculata

Andrographis paniculata (Acanthaceae) is an annual herb. It is found in Sri Lanka and throughout the plains of India specially Maharashtra, Karnataka, Uttar Pradesh, Tamilnadu, Orissa. Various medicinal properties like choleretic, antidiarrhoeal, immunostimulant and antiinflammatory have been attributed to this plant in the traditional system of Indian medicine[1-5]. Further reported activities are hepatoprotective, antimalarial, antihypertensive, antipyretic, antithrombotic and antidote for snake bites. The present investigation was undertaken to find out the antibacterial potentiality of the ethanol extract of the aerial part against some Gram positive and Gram negative bacteria.

The plants Andrographis paniculata were collected from the Similipal Biosphere Reserve, Mayurbhanj, Orissa and identified at Botanical Survey of India, Kolkata (Ref no.CNH/l-l (59)/2006/Tech.ll dated. 27.10.06). The aerial part of Andrographis paniculata were shade dried followed by drying in hot air oven.