

Anti HIV and Antibacterial Activities of Some Disubstitutedquinazolones and their Bio-isostere Disubstitutedthienopyrimidones

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Anti HIV and antibacterial activity of some 2,3-disubstitutedquinazolones, 2-substituted-[1,3,4]-thiadiazoloquinazolones and their bio-isostere-[1,3,4]-thiadiazolothienopyrimidones were determined. Among the compounds tested for antiHIV activity, the 2-methyl -[1,3,4]-thiadiazolo [2,3-*b*]-6,7,8,9-tetrahydrobenzo(*b*)thieno-[3,2-*e*]-pyrimidin-5(4*H*)-one (USP/VA-2) gave maximum protection against HIV-2 (ROD). The compounds 2-(4-methoxyphenylamino)-[1,3,4]-thiadiazolo-[2,3-*b*]-6,7-dimethylthieno-[3,2-*e*]-pyrimidin-5(4*H*)-one (USP/VA-1) and USP/VA-2 were exhibited comparable antibacterial activity with the standard amoxycill in against *Escherichia coli*.

Acquired immunodeficiency syndrome (AIDS) is a fatal pathogenic disease caused by a retrovirus called human immunodeficiency virus (HIV)^{1,2}. In the search for more selective and effective agents for the treatment of this fatal disease, various heterocyclic compounds have been designed to inhibit replication of HIV^{3,4}. Quinazolines and condensed quinazolines are reported^{5,6} to possess potential antiHIV activity apart from other biological activities such as antibacterial⁷, antifungal⁸, antitubercular⁹ and anticancer¹⁰. In spite of a large number of quinazolines and condensed quinazolines have been prepared and studied for various biological activities, the (1,3,4)-thiadiazoloquinazolines have received only scant attention¹¹. Their bio isostere (1,3,4)-thiadiazolothienopyrimidines are relatively unexplored¹². Encouraged by these findings, it was thought worthwhile to synthesize some 2,3-disubstitutedquinazolones, 2-substituted-[1,3,4]-thiadiazolo quinazolones and 2-substituted-[1,3,4]-thiadiazolothieno pyrimidines and investigate their antiHIV and antibacterial activities.

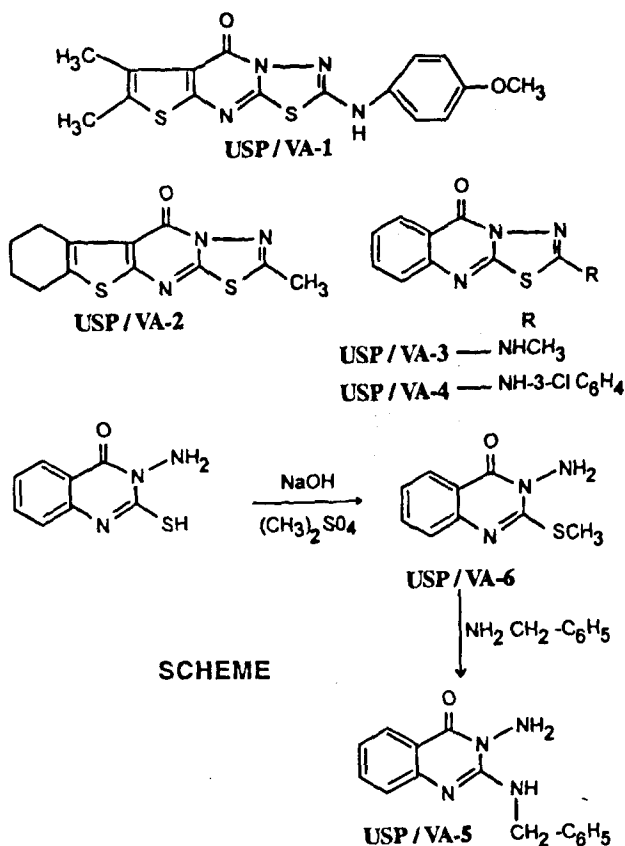
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Earlier we have reported the synthesis of 2-substituted-[1,3,4]-thiadiazolo-[2,3-*b*]-6,7-disubstitutedthieno-[3,2-*e*]-pyrimidin-5(4*H*)-ones (USP/VA-1 and USP/VA-2)¹³ and 2-substituted-[1,3,4]-thiadiazolo-[2,3-*b*]-quinazolin-5-(4*H*)-ones (USP/VA-3 and USP/VA-4)¹⁴. The 3-amino-2-benzylaminoquinazolin-4(3*H*)-one (USP/VA-5) was synthesized by treating benzylamine with 3-amino-2-methylthioquinazolin-4(3*H*)-one (USP/VA-6) which in turn was synthesized from 3-amino-2-mercaptoquinazolin-4(3*H*)-one Scheme-I. The starting material, 3-amino-2-mercaptoquinazolin-4(3*H*)-one was synthesized as reported earlier¹⁴. Compounds synthesized were characterized by spectral (IR, NMR and mass) data and elemental analysis.

EXPERIMENTAL

All the melting points are uncorrected. IR spectra were recorded in KBr on a Perkin Elmer-841 Grating spectrometer (γ_{\max} in cm^{-1}); mass spectra on a Varian Atlas CH-7 mass spectrometer at 70 eV; and ¹H NMR spectra on a varian A-60 or EM-360 spectrometer at 60 MHz

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(chemical shifts in δ ppm) using TMS as internal standard.

Synthesis of 3-Amino-2-methylthioquinazolin-4(3H)-one USP/VA-6:

A solution of 3-amino-2-mercaptoquinazolin-4(3H)-one (1.93 g; 0.01 mol) in sodium hydroxide (20%, 10 ml) was obtained by warming on a water bath. It was clarified by filtration while in warm condition, cooled and treated with dimethylsulphate (1.26 g; 0.01 mol) under constant stirring. The solution was stirred at room temperature for 12 h. The solid obtained was filtered, washed with cold water, dried and recrystallized (Table 1).

Synthesis of 3-Amino-2-benzylaminoquinazolin-4(3H)-one USP/VA-5:

A mixture of benzylamine (5.35 g; 0.05 mol) and 3-amino-2-methylthioquinazolin-4(3H)-one (2.07 g; 0.01 mol) was heated at 80° for 36 h. The reaction mixture was cooled and triturated with petroleum ether (60-80). The solid obtained was filtered, washed with petroleum ether (60-80), dried and recrystallized (Table 1).

Anti-HIV Activity:

Five compounds (USP/VA 1-5) were tested for their anti HIV activity. The MT-4 cells were grown in RPMI-1640 DM (Dutch modification) medium (Flow Laboratories, Irvine, Scotland), Supplemented with 10% (v/v) heat-inactivated fetal calf serum (FCS) and 20 μ g/ml gentamicin (E. Merck, Darmstadt, Germany). The cells were maintained at 37° in a humidified atmosphere of 5% CO₂ in air. Every 3-4 days, cells were spun down and seeded at 3 x 10⁵ cells/ml in new cell culture flasks. At regular time intervals, the MT-4 cells were analyzed for the presence of mycoplasma and consistently found to be mycoplasma-free. HIV-1 (strain HTLV-III_B LAI)¹⁵ and HIV-2 (strain LAV-2ROD)¹⁶ were obtained from the culture supernatant of HIV-1 or HIV-2 infected MT-4 cell lines¹⁷. The virus titer of the supernatant was determined in MT-4 cells. The virus stocks were stored at -70° until used.

Flat bottom, 96-well plastic microtiter plates (Falcon, Becton Dickinson, Mountain view, CA) were filled with 100 μ l of complete medium using a Titertek Multidrop dispenser (Flow Laboratories). This eight-channel dispenser could fill a microtiter tray in less than 10 s. Subsequently, stock solutions (10 x final test concentration) of compounds were added in 25 μ l volumes to two series of triplicate wells so as to allow simultaneous evaluation of their effects on HIV-and mock-infected cells. Serial five-fold dilutions were made directly in the microtiter trays using a Biomek 1000 robot (Beckman). Untreated control HIV- and mock-infected cell samples were included for each compound.

Fifty microlitres of HIV at 100 CCID₅₀ medium was added to either infected or mock-infected part of a microtiter tray. Exponentially growing MT-4 cells were centrifuged for 5 min at 140 x g and the supernatants were discarded. The MT-4 cells were resuspended at 6 x 10⁵ cells/ml in a flask which was connected with an autoclavable dispensing cassette of a Titertek Multidrop dispenser. Under slight magnetic stirring 50 μ l volumes were then transferred to the microtiter tray wells. The outer row wells were filled with 200 μ l of medium. The cell cultures were incubated at 37° in a humidified atmosphere of 5% CO₂ in air. The cells remained in contact with the test compounds during the whole incubation period. Five days after infection the viability of mock and HIV-infected cells were examined spectrophotometrically by the MTT method.

TABLE 1 : CHARACTERIZATION DATA OF COMPOUNDS USP/VA-5 AND USP/VA-6

Compound Code	m.p. (solvent)*	Yield %	Mol. Formula (Mol. Wt)**	¹ HNMR (δ ppm) in DM SO - d ₆
USP/VA-6	155 - 157 (Chloroform-ethanol)	90	C ₉ H ₉ N ₃ OS (207)	2.51 (s,3H,SCH ₃), 6.6 (s,2H,NH ₂) D ₂ O exchangeable 7.5-7.8 (m,4H,Ar-H)
USP/VA-5	170-171 (Chloroform-ethanol)	72	C ₁₆ H ₁₄ N ₄ O (266)	4.3 t,1H,NHCH ₂ C ₆ H ₅) D ₂ O exchangeable 4.9 (s, 2H,-CH ₂) 5.3 (s, 2H,NH ₂) D ₂ O exchangeable 7.4-7.8 (m,9H,Ar-H)

*All the compounds gave satisfactory elemental analysis ** Mol Wt. Determination by mass spectra.
IR (KBr) spectra of both compounds showed characteristic peaks in cm⁻¹ for NH (3200 - 3400) CO - N (1680 - 1700), C = N (1640) and C = C (1600)

MTT assay:

The MTT assay is based on mitochondrial the reduction of the yellow coloured 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) on drial (Sigma Chemical Co., St. Lous, MO) by mitochondrial dehydrogenase of metabolically active cells to a blue formazan which can be measured spectrophotometrically. Therefore, to each well of the microtiter plates 20 μl of a solution of MTT (7.5 mg/ml) in phosphate-buffered saline was added using the Titertek Multidrop. The trays were further incubated at 37° in a CO₂ incubator for 1 h. A fixed

volume of medium (150 μl) was then removed from each cup using M96 washer (ICN flow) without disturbing the MT-4 cell cluster containing the formazan crystals.

Solubilization of the formazan crystals was achieved by adding 100 μl 10% (v/v) Triton X-100 in acidified isopropanol (2 ml concentrated HCl per 500 ml solvent) using the M96 washer. Complete dissolution of the formazan crystals could be obtained after the trays had been placed on a plate shaker for 10 min. Finally, the absorbances were read in a eight-channel computer-controlled photometer (Multiskan MCC, ICN Flow) at two wavelengths

TABLE 2 : ANTI HIV ACTIVITY

Code	Strain	EC ₅₀ ^a (μg/ml)	CC ₅₀ ^b (μg/ml)	Max Protection (%)
USP/VA-1	IIIB	>27	27.5	1
USP/VA-2	IIIB	>64	63.5	23
USP/VA-2	IIIB	>48	48	0
USP/VA-2	ROD	>75	75	41
USP/VA-2	ROD	>43	43.2	19
USP/VA-3	IIIB	>50	50.5	3
USP/VA-4	IIIB	>30	30.4	1
USP/VA-5	IIIB	>25	24.7	3

^aEffective concentration of compound, achieving 50% protection of MT-4 cells against the cytopathic effect of HIV.

^bCytotoxic concentration of compound, required to reduce the viability of normal uninfected MT-4 cells by 50%

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TABLE 3 : ANTIBACTERIAL ACTIVITY OF TEST COMPOUNDS

Microorganism	Test Compounds						Standard amoxicillin)
	USP/VA1	USP/VA2	USP/VA3	USP/VA4	USP/VA5	USP/VA6	
<i>Escherichia coli</i>	18	21	13	16	9	7	28
<i>Staphylococcus aureus</i>	15	16	14	15	6	5	32
<i>Salmonella typhi</i>	13	12	11	10	8	6	30
<i>Vibrio cholerae</i>	14	17	11	13	5	9	27

Numbers indicate zone of inhibition in nm of various test compounds and standard amoxicillin against different test bacteria.

(540 and 690 nm). The absorbance measured at 690 nm was automatically subtracted from the absorbance at 540 nm, so as to eliminate the effects of non-specific absorption. Blanking was carried out directly on the microtiter plates with the first column wells which contained all reagents except the MT-4 cells.

All data represent the average values for a minimum of three wells. The 50% cytotoxic concentration (CC_{50}) was defined as the concentration of compound that reduced the absorbance (OD_{540}) of the Mock-infected control sample by 50%. The percent protection achieved by the compound in HIV-infected cells was calculated by the following formula:

$$\frac{(OD_T)_{HIV}}{(OD_C)_{mock}} - \frac{(OD_C)_{HIV}}{(OD_C)_{mock}}$$

Where $(OD_T)_{HIV}$ is the optical density measured with a given concentration of the test compound in HIV infected cells; $(OD_C)_{mock}$ is the optical density measured for the control untreated mock infected cells; all OD values determined at 540 nm. The dose achieving 50% protein according to the above formula was defined as the 50% effective concentration (EC_{50}). The results are presented in Table 2.

Antibacterial Activity:

The compounds (USP/VA 1-6) were investigated for their antibacterial activity by Agar cup-plate method¹⁸ at a concentration of 100 µg/ml using DMF as a solvent against the following organisms, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi* and *Vibrio cholerae* employing ampicillin (100 µg/ml) as a reference standard. The zone of inhibition was measured and presented in Table 3.

RESULTS AND DISCUSSION

The results of antiHIV activity (Table 2), show that compounds USP/VA-2 exhibited maximum 41% protection against HIV-2 (ROD) and 23% protection against HIV-1 (IIIB) at subtoxic concentration. Rest of the compounds exhibited very little protection.

From the results of antibacterial activity (Table 3) the compounds USP/VA-1 and USP/VA-2 were found to be comparably active with the standard against *E.coli*. The thiadiazolothienopyrimidines (USP/VA-1 and USP/VA-2) showed better activity against all the microorganisms tested. All the thiadiazoloquinazolones (USP/VA-3 and USP/VA-4) exhibited appreciable activity, while the 2,3-disubstitutedquinazolines (USP/VA-5 and USP/VA-6) exhibited very minimal activity against all the bacteria tested. Thus, these study gave an indication that further modification in thiadiazolothienopyrimidines series may yield more potent compound.

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