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AntiHIV and Antibacterial Activities of 2-Substituted Thiadiazolo Quinazolines

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Accepted 20 December 2002

Revised 18 November 2002

Received 21 December 2001

AntiHIV and antibacterial activities of 2-substituted-(1,3,4)-thiadiazolo (2,3-*b*) quinazolin-5(4*H*)-ones, were determined. Among the compounds tested for antiHIV activity, the compound VA5 gave maximum protection against HIV-2 (ROD). The compound VA2 (at 100 μg/ml) exhibited equivalent antibacterial activity with the standard ciprofloxacin (at 10 μg/ml) against *Klebsiella pneumoniae, Bacillus subtilis, Staphylococcus epidermitis, Shigella flexnari and Citrobacter ferundi.*

Recent literature is enriched with findings about the synthesis and pharmacological screening of quinazolines and condensed quinazolines[1]. The thiadiazolo quinazoline nucleus is associated with diverse pharmacological activities such as antibacterial[2,3], antifungal[4], phosphodiesterase inhibitory[5], antiinflammatory[6], platelet aggregation inhibitory[7] and antihypertensive[8,9]. Inspite of various condensed thiadiazolo quinazoline systems have been synthesized and studied for biological activities, the synthesis of (1,3,4)-thiadiazolo-(2,3-*b*)-quinazolines have received only scant attention[10]. Infact the first report on the synthesis of (1,3,4)-thiadiazolo-(2,3-*b*) quinazoline appeared in 1970 and very few reports have appeared since then. Earlier we have reported the synthesis, antiHIV and antibacterial activities of some thiadiazolo quinazolones and its bioisostere thriadiazolo thienopyrimidines[11,12]. In continuation of this work herein we report the antiHIV and antibacterial activities of a few 2-substituted (1,3,4)-thiadiazolo-(2,3-*b*)-quinazolin-5(4*H*)-ones. The title compounds (fig. 1) were prepared using methods that were earlier reported from our laboratory[11].

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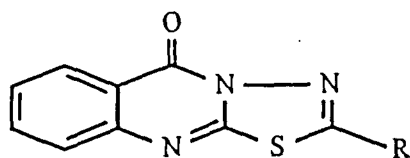
Melting points were determined in open capillary tubes on a Thomas Hoover apparatus and are uncorrected. IR spectra were recorded in KBr on a Perkin Elmer-841 grating spectrophotometer (cm^{-1}), mass spectra on a Varian Atlas CH-7 mass spectrometer at 70 eV. Elemental analysis was performed on a Carlo erba 1108.

The starting material 3-amino-2-mercapto quinazolin-4(3*H*)-one was prepared[11] by adding carbondisulphide (1.6 ml, 0.026 mol) and aqueous sodium hydroxide (1.2 ml, 20 mol solution) dropwise simultaneously, to a vigorously

stirred solution of methylanthranilate (3.02 g, 0.02 mol) in dimethylsulfoxide (10 ml) at room temperature. After 30 min,



Compd.		R
VA-1	-	H
VA-2	-	$NH\text{-}CH_2\text{-}CH{=}CH_2$
VA-3	-	NHC_6H_5
VA-4	-	$NH\text{-}3\text{-}CH_3C_6H_4$
VA-5	-	$NH\text{-}4\text{-}OCH_3C_6H_4$

Fig. 1: Structures of the title compounds.

dimethyl sulphate (2.5 g, 0.02 mol) was added dropwise under cooling with an ice bath. Stirring was continued for 3 h, the reaction mixture was poured into ice-water and then it was extracted with chloroform. The solvent was removed by distillation under reduced pressure. Thus the obtained crude methyl N-(2-methoxycarbonylphenyl) dithiocarbamate was used for further reaction without purification. Hydrazine hydrate (8.6 g, 0.2 mol) was added dropwise to a stirred methyl N-(2-methoxycarbonylphenyl) dithiocarbamate (4.82 g, 0.02 mol) in cold condition. After the completion of addition, stirring was continued for 1.5 h at 50° and the mixture was poured into ice-water. The solid so obtained was filtered, washed with water, dried and recrystallized from dimethylformamide and ethanol. Yield: 1.72 g (90%); M.P.: 236-237°; **IR** (KBr): 3300, 3220 (NH_2), 2560 (SH), 1680 (C=0) cm^{-1}; ^{1}**H NMR** ($CDCl_3$) δ ppm: 3.21(s,1H,SH), 5.12 (s,2H,NH_2,$D_2$0 exchangeable), 7.14 (m, 4H, ArH); MS(m/e):

TABLE 1: ANTIHIV ACTIVITY.

Compd. Code	Strain	CC_{50}[b] (μg/ml)	EC_{50}[a] (μg/ml)	SI	Max. protection (%)
VA1	III B	>113	113	<1	5
VA1	III B	>51.1	51.1	<1	0
VA1	ROD	>63.7	63.7	<1	9
VA2	III B	>105	105	<1	3
VA2	III B	>75.8	75.8	<1	14
VA2	III B	>19.6	19.6	<1	0
VA2	ROD	>97.1	97.1	<1	4
VA3	III B	>35.3	35.3	<1	9
VA3	ROD	>42.9	42.9	<1	2
VA4	III B	>54.5	54.5	<1	4
VA4	III B	>26.8	26.8	<1	7
VA4	ROD	>32	32	<1	0
VA5	III B	>125	>125	X1	9
VA5	III B	>73.3	>73.3	X1	7
VA5	ROD	>125	>125	X1	15
VA5	ROD	>69.8	69.8	<1	4

[a]Effective concentration of compound, achieving 50% protection of MT-4 cells against the cytopathic effect of HIV. [b]Cytotoxic concentration of compound required to reduce the viability of normal uninfected MT-4 cells by 50%. Cytopathic effect – Viruses cause cell degeneration or cell death which can be seen by microscopical examination of cultures. Cell degeneration is manifested by certain pathological changes.

193 (M^+); Anal ($C_8H_7N_3OS$) C, H, N.

The title compound, 1,3,4-thiadiazolo(2,3-*b*) quinazolin-5(4*H*)-one (VAI) was prepared[11] by taking a mixture of 3-amino-2-mercapto quinazolin-4(3*H*)-one (3.86 g; 0.02 mol) and a drop of conc. Sulphuric acid in excess of triethylorthoformate (25 ml), it was refluxed for 10 h and the excess of triethylorthoformate was distilled under reduced pressure, the solid obtained was filtered, dried and recrystallised from chloroform. Yield: 1.36 g (67%); M.P.: 231-233°; IR (KBr): 1680 (C=0), 1650 cm^{-1} (C=N); ^{1}H NMR ($CDCl_3$); δ ppm: 6.6-7.0 (m, 5H, Ar-H); MS(m/e): 203 (M^+); Anal ($C_9H_5N_3OS$) C, H, N. Similarly the compounds VA2-VA5 were prepared.

The compounds were tested for antiHIV activity against replication of HIV-I (III B) and HIV-2 (ROD) in MT-4 cells[12]. The MT-4 cells were grown in RPMI-1640 DM (Dutch modification) medium (Flow lab, Irvine, Scotland), supplemented with 10% (v/v) heat inactivated fetal calf serum and 20 μg/ml gentamicin (E. Merck, Darmstadt, Germany). HIV-1 (III B) and HIV-2 (ROD) were obtained from the culture supernatant of HIV-I infected MT-4 cell lines and the virus stocks were stored at -70° until used. The antiHIV assay was carried out in microtiter plates filled with 100 μl of medium and 25 μl volumes of compounds in triplicate so as to allow simultaneous evaluation of their effects on HIV and mock infected cells. Fifty microlitres of HIV at 100 $CCID_{50}$ medium was added to either infected or mock infected part of microtiter tray. The cell cultures were incubated at 37° in a humidified atmosphere of 5 % CO_2 in air. Five days after infection the viability of mock and HIV-infected cells were examined spectrophotometrically by the MTT method. The effective dose of compound achieving 50% protection of MT-4 cells against the cytopathic effect (Viruses cause cell degeneration or cell death which can be seen by microscopical examination of cultures. Cell degeneration is manifested by certain pathological changes.) of HIV (EC_{50}), the cytotoxic dose of compound, required to reduce the viability of normal uninfected MT-4 cells by 50 % (CC_{50}) and selective indices (SI) or ratio of CC_{50} to EC_{50} are determined.

The results of antiHIV activity (Table 1) show that compound VA5 exhibited maximum 15% protection against HIV-2 (ROD) and VA2 exhibited 14% protection against HIV-1 (III B) at subtoxic concentration. Rest of the compounds exhibited very little protection.

The compounds (VA1-VA5) were investigated for antibacterial activity by agar cup-plate method[13] at a concentration of 100 μg/ml using DMF as a solvent against the following organisms, *Vibreo cholerae, Klebsiella pneumoniae, Citrobacter ferundi, Salmonella typhi, Staphyllococcus aureus, Staphylococcus epidermitis, Shigella flexnari,* and

TABLE 2: ANTIBACTERIAL ACTIVITY[a] OF TEST COMPOUNDS BY AGAR CUPPLATE METHOD.

Micro-organism	Test Compounds (100 μg/ml)					
	VA1	VA2	VA3	VA4	VA5	Ciprofloxacin (10 μg/ml)
V. cholerae	15	18	20	17	15	23
K. pneumoniae	16	21	21	16	29	27
B. subtilis	20	26	19	17	19	28
E. coli	19	20	18	16	17	24
C. ferundi	18	25	20	16	19	26
S. typhi	18	19	15	20	18	29
S. aureus	16	21	18	23	17	25
S. epidermitis	19	26	15	16	25	23
S. flexnari	18	24	17	17	15	22
P. aeruginosa	17	20	19	16	19	25

[a]Numbers indicate zone of inhibition in mm of various test compounds and standard ciprofloxacin against different bacteria.

Pseudomonas aeruginosa employing ciprofloxacin (10 μg/ml) as a reference standard. The zone of inhibition was measured and presented in Table 2.

The results in Table 2 indicated that all the compounds exhibited appreciable antibacterial activity, while the compounds VA2 exhibited equivalent activity with the standard ciprofloxacin against *K. pneumoniae, B. subtilis, C. ferundi, S. epidermitis and S. flexnari*, the compound VA5 exhibited equivalent activity with the standard against *K. pneumoniae* and *S. epidermitis*.

ACKNOWLEDGEMENTS

We are grateful to the management S. B. College of Pharmacy, Sivakasi and Rega Institute for Medical Research, Belgium for providing necessary facilities to carryout this research work.

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Four Simple Spectrophotometric Determinations of Lisinopril in Pure State and in Tablets

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Accepted 21 December 2002
Revised 20 November 2002
Received 8 July 2002

Four simple and sensitive procedures (methods A, B, C and D) for the assay of lisinopril in pure form and formulations are described. Methods A and B are based on the condensation of lisinopril (acyclic imino acid) with ninhydrin (indane-1,2,3-trione hydrate) in the presence of ascorbic acid (method A, λ_{max} 560 nm) or ascorbic acid (method B, λ_{max} 520 nm). Method C is based on the initial formation of water insoluble adduct involving lisinopril and phosphomolybdic acid, followed by release of phosphomolybdic acid from the adduct with acetone and color development with cobalt nitrate–ethylenediaminetetraacetic acid disodium salt complex (λ_{max} 840 nm). Method D is based on the formation of colored radical anion on treating lisinopril with 2, 3-dichloro,5,6-dicyano-1,4-benzoquinone (λ_{max} 460 nm). The variable parameters in all these methods have been optimized. The results were statistically validated.

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