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AntiHIV, Antibacterial and Antifungal Activities of Some 2,3-Disubstituted Quinazolin-4(3*H*)-ones

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The title compounds 2-mercapto-3-(substitutedmethylamino) quinazolin-4(3*H*)-ones were synthesized by condensing the active hydrogen atom of 3-amino group of 3-amino-2-mercapto quinazolin-4(3*H*)-one with formaldehyde and the desired amines. Investigation of antimicrobial activity of the compounds was performed by agar dilution method against 6 pathogenic bacteria, 3 pathogenic fungi and antiHIV activity against replication of HIV-1 (IIIB) and HIV-2 (ROD) in MT-4 cells. The compounds exhibited significant antibacterial and antifungal activities.

Quinazolines and condensed quinazolines are reported to show a variety of biological activities, such as antibacterial[1], antifungal[2] and antiHIV[3]. Mannich bases are reported to possess potent antibacterial, antifungal and antiHIV activities[4]. In view of these facts and as a continuation of our earlier efforts carried out in our laboratory[3,5], 2-mercapto-3-(substitutedmethylamino) quinazolin-4(3*H*)-ones were synthesized. The title compounds were synthesized by condensing the active hydrogen atom of 3-amino group of 3-amino-2-mercapto quinazolin-4(3*H*)-one with formaldehyde and the desired amines [Mannich reaction]. The starting material 3-amino-2-mercapto quinazolin-4(3*H*)-one was prepared from anthranilic acid using methods reported[6] from our laboratory. The title compounds (fig.1) were screened for antibacterial, antifungal activity by agar dilution method and antiHIV activity against HIV-1(III B), HIV-2(ROD) in MT-4 cells.

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Melting points were determined in open capillary tubes on a Thomas Hoover melting point apparatus and are uncorrected. IR spectra were recorded in KBr on a Perkin Elmer-841 grating spectrometer (cm^{-1}), Mass spectra on a Varian Atlas CH-7 mass spectrometer at 70 ev and NMR Spectra on a Varian A-60 or EM-360 spectrometer, using TMS as internal standard. Elemental analysis was performed on a Carlo Erba 1108 CHN analyzer.

The starting material, 3-amino-2-mercapto quinazolin-4(3*H*)-one was synthesized by the following method. To a vigorously stirred solution of methyl anthranilate (3.02 g, 0.02 mol) in dimethyl sulfoxide (10 ml) at room temperature, carbon disulphide (1.6 ml, 0.026 mol) and sodium hydroxide (1.2 ml, 20 mol) were added during 30 min. After 30 min, dimethyl sulfate (2.5 g, 0.02 mol) was added drop wise at 5-

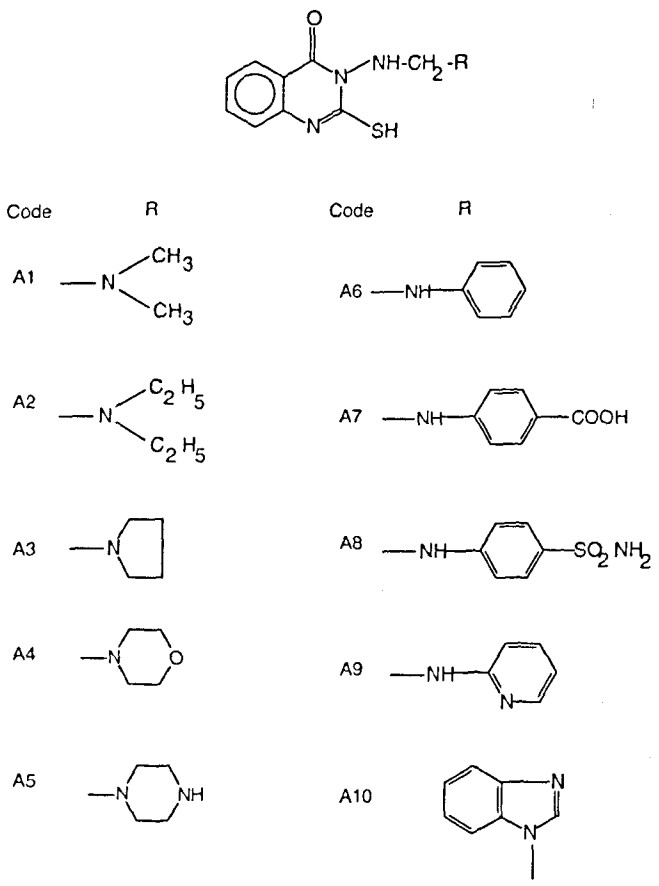

Fig 1: Structures of Synthesized Compounds.

10°. Stirring was continued for 3 h, the reaction mixture was then poured into ice water and extracted with chloroform. The solvent was removed by distillation under reduced pressure, resulting in methyl-N-(2-methoxycarbonyl) dithiocarbamate, which was used for further reaction without purification. Hydrazine hydrate (0.2 mol, 80%) was added drop wise with stirring to a stirred methyl-N-(2-methoxycarbonylphenyl) dithiocarbamate at 5-10°. After the completion of addition, stirring was continued for 1.5 h at 50° and then it was poured into ice water, the solid obtained was filtered, washed with water, dried and recrystallized from dimethylformanide -ethanol mixture. Yield: 3.4 g (90%); m.p.: 236-237°; IR (KBr): 3300, 3220 (NH_2), 2990 (CH), 2560 (SH), 1680 (C=O); ^{1}H NMR ($CDCl_3$) δ ppm: 3.21 (s, 1H, SH), 5.12 (s, 2H, NH_2, D_2O exchangeable), 7.14 (m, 4H, ArH); MS (m/e): 193 (M^+); Anal. ($C_8H_7N_3OS$) C, H, N.

The title compound 2-mercapto-3-(N,N-dimethylaminomethylamino) quinazolin-4(3*H*)-one (A1) was synthesised by adding a mixture of formalin (1 ml) and dimethylamine (0.23 g, 0.005 mol) drop by drop with stirring to a slurry of 3-amino-2-mercapto quinazolin-4(3*H*)-one (0.96 g, 0.005 mol) in dimethyl formamide (15 ml). The reaction mixture was then heated at 50° with stirring for 30 min. After cooling, reaction mixture was poured into ice water, the solid obtained was filtered, washed with water, dried and recrystallized from alcohol-chloroform mixture. Yield: 0.94 g (75%); m.p: 140-142°; IR (KBr); 3380 (NH), 3050 (CH), 2850 (CH), 2500 (SH), 1700 (C=0); ^{1}H NMR ($CDCl_3$) δ ppm: 1.9 (s, 2H, CH_2), 2.1(s, 6H, $2CH_3$), 7.1 (m, 4H, Ar-H), 8.6 (t, 1H, NH); MS (m/e): 250 (M^+); Anal ($C_{11}H_{14}N_4OS$) C, H, N. Compounds (A2-A10) were prepared similarly.

The compounds (A1-A10) were tested for antiHIV activity against replication of HIV-1 (III B) and HIV-2 (ROD) in MT-4 cells[3]. 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-1 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 antiHIV data (Table 1) indicates that the compound A10 showed maximum 31% and the compound A6 showed maximum 25% protection against HIV-1 (III B); the compound A9 showed 27% protection against HIV-2 (ROD) while the rest of compounds exhibited very little protection at sub toxic concentration.

Antibacterial and antifungal activities were carried out by agar dilution method[7], all bacteria were grown on Muller-Hinton Agar (Hi-media) plates (37°, 24 h) and fungi were grown on sabouraud dextrose agar (Hi-media) plates (26°,

48-72 h). The minimum inhibitory concentration (MIC) was considered to be the lowest concentration that completely inhibited the growth on agar plates, disregarding a single colony or faint haze caused by the inoculum. The MIC values of the synthesized compounds against 6 bacteria and 3 fungi are presented in Table 2. Also the activity of reference compounds, norfloxacin and clotrimazole were included.

The results of antibacterial activity (Table 2) revealed that all the test compounds showed moderate activity against the tested bacteria. The compound A8 exhibited good activity against *E. tarda*, *B. subtilis*, *Proteus vulgaris*. The compound A7 showed good activity against, *P. vulgaris*, *E. tarda* and *S. albus*; and the compound A1 exhibited good activity against *B. subtilis*. The compound A8 was found to be the

TABLE 1: ANTIHIV ACTIVITY OF TEST COMPOUNDS (A1-A10)

Code	Strain	CC^{a}_{50} (μg/ml)	EC^{b}_{50} (μg/ml)	SI[c]	Max. Prot. %
A1	III B	>63.0	63.0	>1	6
	III B	>48.2	48.2	>1	8
	ROD	>54.1	54.1	>1	2
A2	III B	>125	>125	@1	0
	III B	>125	>125	@1	0
	ROD	>125	>125	@1	4
A3	III B	>12.5	12.5	>1	19
	III B	>24.9	24.9	>1	4
	ROD	>13.8	13.8	>1	21
	ROD	>24.6	24.6	>1	5
A4	III B	>15.2	15.2	>1	5
	III B	>12.8	12.8	>1	3
	ROD	>16.1	16.1	>1	0
A5	III B	>77.4	77.4	>1	0
	III B	>73.3	73.3	>1	3
	ROD	>89.4	89.4	>1	5
A6	III B	>62.8	62.8	>1	25
	III B	>26.4	26.4	>1	19
	ROD	>62.6	62.6	>1	7
	ROD	>37.5	37.5	>1	20
A7	III B	>125	>125	@1	9
	III B	>125	>125	@1	11
	ROD	>125	>125	@1	4
A8	III B	>125	>125	@1	3
	III B	>125	>125	@1	2
	ROD	>125	>125	@1	1
A9	III B	>125	>125	>1	11
	III B	>65.1	65.1	>1	3
	ROD	>80.7	80.7	>1	27
	ROD	>56.9	56.9	>1	10
A10	III B	>125	>125	@1	2
	III B	>125	>125	@1	31
	ROD	>125	>125	@1	0

[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%. [c]Selective index or ratio of CC_{50} to EC_{50}.

TABLE 2: ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES MIC VALUES (μG/ML)[a]

Microorganisms/drugs	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	Std
E. tarda	156	78	78	78	312	78	39	39	78	78	97
S. albus	156	78	312	78	156	78	39	156	78	156	2
S. enteritidis	78	156	78	78	312	78	156	78	78	312	1
B. Subtilis	39	78	156	78	156	78	312	39	156	78	1
Shigella sonnei	78	78	156	78	312	78	312	78	156	78	9
Proteus vulgaris	78	78	156	78	78	78	39	9	156	78	1
Candida albicans	78	39	78	156	78	78	39	78	312	78	0
Aspergillus niger	78	19	78	78	78	156	19	78	78	78	2
M. audouinii	78	156	78	19	312	78	156	78	78	19	19

[a] MIC, minimum inhibitory concentration.

most active antibacterial agent. In general the presence of p-carboxy and p-sulphonamide substitution showed better activity. All the compounds showed significant antifungal activity (Table 2). When compared to clotrimazole the compounds A4 and A10 have shown equipotent activity (MIC 19.53 μg/ml) against the dermatophyte *M. audouinii*. The compound A2 showed good activity against *Candida albicans* and *Aspergillus niger*.

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