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SYNTHESIS AND ANTIVIRAL ACTIVITY OF SOME NOVEL 2–SUBSTITUTED, 3–(6–ETHYL, 4–AMINO, 5–(4–CHLOROPHENYL)– PYRIMIDIN–2–YL)] QUINAZOLIN–4 (3H)–ONES

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ABSTRACT

2–substituted benzoxazin–4–ones were condensed with pyrimethamine to form the 2.3–disubstituted quinazolin–4(3H)–ones. Their chemical structures were elucidated by means of spectral analysis (IR, NMR and MS). Synthesized compounds were evaluated for antirival activity against HSV–1 and 2 in HEL cells, influenza B virus in MDCK cells and SARS virus in Vero E6 cells. The compound 2–phenyl, 3–[(6–ethyl, 4–amino, 5–(4–chlorophenyl) pyrimidin–2–yl)] quinazolin–4(3H)–one (**3**) have minimum inhibitory concentration of 48 µg/mL against herpes simplex virus–1. Compounds (**1**) and (**3**) were active against influenza B virus in MDCK cells.

Key words: Quinazolin–4(3H)–one, Pyrimethamine, Antiviral activity.

INTRODUCTION

Quinazolin–4(3H)–one is a versatile lead molecule for the designing of potential bioactive agents. Quinazolin–4(3H)–one derivatives were reported to posses sedative[1], anticonvulsant[2], antifungal and antibacterial[3], anti–HIV[4,5] and anticancer[5] activities. Schiff and Mannich bases of isatin with trimethoprim and pyrimethamine were synthesized and screened for anti–HIV activity. Some of their derivatives showed significant anti–HIV activity[6,7].s In recent years, much attention has been devoted in search of effective chemotherapeutic agents for the treatment of fatal diseases like AIDS, SARS etc. We have synthesized some novel heterocyclic compounds and evaluated for antiviral activity. Some of these derivatives exhibited significant activity against HIV and vaccinia viruses[9–12].

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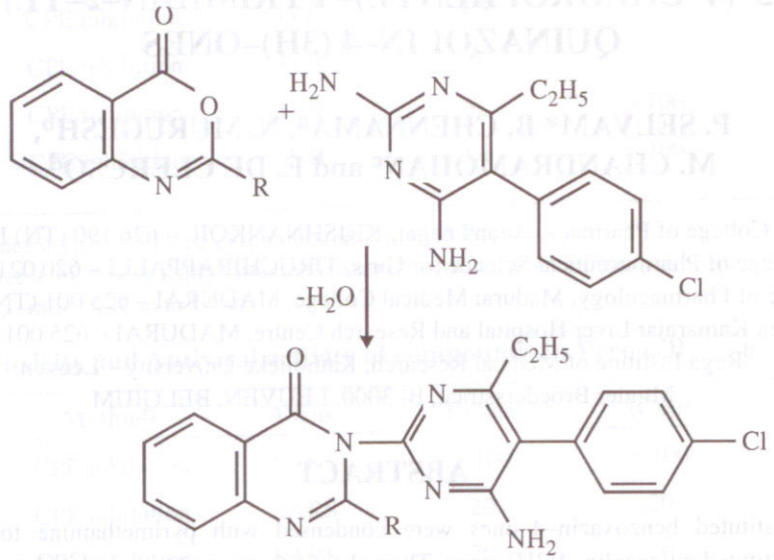
The present work involves the synthesis of some 2,3–disubstituted quinazolin–4(3H)–one derivatives by condensation of 2–substituted 1,3–benzoxazin–4–ones[8] and pyrimethamine (**Scheme–1**). The synthesized compounds were evaluated for the antiviral activities against



R = CH_3, C_2H_5, C_6H_5 for **(1)**, **(2)** and **(3)** respectively

Scheme 1

Herpes simplex virus–1 and 2 in HEL cells, influenza virus B in MDCK cells and SARS virus Vero E6 cells.

EXPERIMENTAL

Melting points were determined by using Thomas Hoover melting point apparatus and are uncorrected. The structures of the synthesized compounds were elucidated by using DOMEM MV 102 FT–IR in KBr disc and ^{1}H NMR was taken on a Bruker AMX (500 MHz) FT–NMR. Mass spectra were obtained on a Varian Atlas CH–7 mass spectrometer at 70 eV. The purity was checked by TLC using silica gel G as stationary phase and $CHCl_3$: CH_3OH (9 : 1) as mobile phase.

Synthesis of 2–substituted, 3–[(6–ethyl, 4–amino, 5–(4–chlorophenyl) pyrimidin–2–yl)] quinazolin–4(3H)–ones

An equimolar (0.01 mol) mixture of 2–substituted–1,3–benzoxazin–4–one and pyrimethamine was refluxed for 6 h in presence of 10 mL glacial acetic acid. The mixture was then cooled at room temperature and poured into crushed ice and solid thus obtained was recrystallised from ethanol.

2–methyl, 3–[4–amino–5–(4–chlorophenyl)–6–ethylpyrimidin–2–yl] quinazolin–4(3H)–one (1)

Yield = 84%, mp 211°C. ^{1}H–NMR (DMSO–d_6) : 1.1 (t, 3H, CH_3), 2.5 (q, 2H, J=6.8 Hz,–CH_2), 3.5 (b, 2H, NH_2), 7.0 (t, 1H, H–7), 7.32 (t, 1H, H–6), 7.5–7.6 (m, 4H, Ar–H), 8.2 (d, 1H, H–5), 8.5 (d, 1H, J=6.8 Hz, H–8); IR (KBr) cm^{-1} : 3455 (NH), 1645 (C=N), 3204 (C–H), 1669 (C=O); El–MS m/z; 391, 859.

2–ethyl,3–[4–amino–5–(4–chlorophenyl)–6–ethylpyrimidin–2–yl]quinazolin–4 (3H)–one (2)

Yield = 87%, mp 210°C, ^{1}H–NMR (DMSO–d_6) : 1.1 (t, 3H, CH_3), 2.5 (q, 2H, J=5.9 Hz, –CH_2), 3.5 (b, 2H, NH_2), 7.0 (t, 1H, J=7 Hz, H–7), 7.32 (t, 1H, H–6), 7.5–7.6 (m, 4H, Ar–H), 8.2 (d, 1H, H–5), 8.5 (d, 1H, J=6.8 Hz, H–8); IR (KBr) cm^{-1} : 3465 (NH) 1645 (C=N), 3210 (C–H) 1672 (C=O); EI–MS m/z:405, 886.

2–phenyl, 3–[4–amino–5–(4–chlorophenyl)–6–ethyl]pyrimidin–2–yl]quinazolin–4(3H)–one (3)

Yield = 88%, mp 169°C, ^{1}H–NMR (DMSO–d_6) : 1.0 (t, 3H, CH_3), 2.5 (q, 2H, J=5Hz, –CH_2), 3.5 (b, 2H, –NH_2), 7.0 (t, 1H, J=7 Hz, H–7), 7.32 (t, 1H, H–6), 7.5 (m, 4H, Ar–H), 7.9 (m, 5H, Ar–H) 8.2 (d, 1H, H–5), 8.7 (d, 1H, H–8); IR (KBr) cm^{-1}: 3393 (NH), 1657 (C=N), 3119 (C–H), 1681 (C=O); El–MS m/z : 453, 93.

Antiviral screening

Antiviral activity of test compounds was tested against HSV–1 and HSV–2 in HEL cell culture[14]. Parameters such as Minimum Inhibitory Concentration (MIC), a concentration of substance required to reduce virus–induced cytopathogenicity to 50% and Minimum Cytotoxic Concentration (MCC), a concentration of substance required to cause microscopically detectable alteration of normal cell were determined.

Table 1. Cytotoxicity and Antiviral activity of compounds in HEL cell

Compound	Minimum Cytotoxic Concentration[a] (μg/mL)	Minimum Inhibitory Concentration[b] (μg/mL)		
		Herpes Simplex Virus–1 (KOS)	Herpes Simplex Virus–1 TKKOS ACV^r	Herpes Simplex Virus–2 (G)
(1)	> 400	> 400	> 400	> 400
(2)	400	> 80	> 80	> 80
(3)	400	48	48	> 48
Brivudin	> 400	0.0153	> 400	80

[a]Required to cause a microscopically detectable alteration of normal cell morphology.
[b]Required to reduce a virus–induced cytopathogenicity by 50%.

Table 2. Cytotoxicity and Antiviral activity of compounds in MDCK cell

Compound	Methods	Virus	EC_{50}a	IC_{50}b	SI[c]
(1)	CPE inhibition	IVB	5.5	> 100	> 18
(2)	CPE inhibition	IVB	6	32	5.3
(3)	CPE inhibition	IVB	6	> 100	> 17
RIBAVIRIN (STD)	CPE inhibition	IVB	1.8	> 100	> 56

[a]Required to reduce a virus–induced cytopathogenicity by 50%.
[b]Required to cause a microscopically detectable alteration of normal cell morphology.
[c]Selectivity index–ratio of IC_{50} to EC_{50}

Table 3. Cytotoxicity and Antiviral activity of compounds in Vero cell

Compound	Methods	Virus	EC_{50}a	IC_{50}b	SI[c]
(1)	CPE inhibition	SARS	> 100	> 100	0
(2)	CPE inhibition	SARS	20	20	1
(3)	CPE inhibition	SARS	20	20	1
RIBAVIRIN (STD)	CPE inhibition	SARS	5	20	4

[a]Required to reduce a virus–induced cytopathogenicity by 50%.
[b]Required to cause a microscopically detectable alteration of normal cell morphology.
[c]Selectivity index–ratio of IC_{50} to EC_{50}

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