Synthesis, Antiviral and Cytotoxic Activity of 6-Bromo-2,3-disubstituted-4(3H)-quinazolinones

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In the present study, a series of 6-bromo-2,3-disubstituted-4(3H)-quinazolinones was synthesized by condensation of 6-bromo-2-substituted-benzoxazin-4-one with trimethoprim, pyrimethamine and lamotrigine. The chemical structures of the synthesized compounds were confirmed by means of IR, 1H-NMR and mass spectral and elemental analysis. The antiviral activity and cytotoxicity of the compounds were tested in E6SM (Herpes simplex-1 KOS, Herpes simplex-1 TK-KOS ACV, Herpes simplex-2 G, Vaccinia virus, Vesicular stomatitis virus, Parainfluenza-3 virus, Reovirus-1, Sindbis virus, Coxsackie virus B4 and Punta Toro virus) and HeLa cell culture (Vesicular stomatitis virus, Coxsackie virus B4 and Respiratory syncytial virus). Investigation of anti-HIV activity was done against replication of HIV-1 (HTLV-III B LAI) in MT-4 cells. 6-Bromo-2-phenyl-3-[4-amino-5-(4-chlorophenyl)-6-ethylpyrimidin-2-yl]-4(3H)-quinazolinone (4) exhibited the most potent antiviral activity with a MIC of 1.92 μg/ml against vaccinia virus in E6SM cell culture. The other compounds did not exhibit antiviral activity nor afford significant cytoprotection to the E6SM and HeLa cell culture when challenged with the viruses. The study implies that 4 may possess activity against Pox viruses including variola. In the anti-HIV study, 6-bromo-2-methyl-3-[4-amino-5-(4-chlorophenyl)-6-ethylpyrimidin-2-yl]-4(3H)-quinazolinone (3) and 6-bromo-2-phenyl-3-[4-amino-5-(4-chlorophenyl)-6-ethylpyrimidin-2-yl]-4(3H)-quinazolinone (4) exhibited the least cytotoxic concentration (0.424, 0.461 μg/ml) which is an index of the infective viability of mock infected MT-4 cells with HIV-1. None of the compounds exhibited significant anti-HIV activity.

Key words quinazoline; antiviral; anti-HIV; cytotoxicity

4(3H)-Quinazolinone derivatives were reported to possess analgesic,1 anti-inflammatory,2 antibacterial, antifungal,3–7 anti-HIV,8,9 antihelminthic,10 antiallergic,11 antitumour,12 anticancer,13 MAO inhibitory14 and central nervous system activities.15 4(3H)-Quinazolinones with 3-substitution has been reported to be associated with antimicrobial properties.16–18 The 3-substitution which were reported are various substituted phenyl ring moieties,19 bridged phenyl rings,20 heterocyclic rings21–23 and aliphatic systems.24 2,3-Substituted-4(3H)-quinazolinones25 were reported to possess antimicrobial properties. The antibacterial, antifungal and anti-HIV screening of semi-derivatized conventional antibacterial agents namely trimethoprim,26,27 sulphadoxine,28,29 norfloxacin,30,31 ciprofloxacin21 and lomefloxacin22 have been reported.

These observation lead to the conception that a new series of 2-methyl/2-phenyl-4(3H)-quinazolinones with 2,4-diaminopyrimidines (trimethoprim and pyrimethamine) and 3,5-diaminotriazine (lamotrigine) substituent in 3rd position would exhibit potential cytoprotective and antiviral activity. The 2,4-diaminopyrimidines selected for the study possess dihydro folate reductase inhibitory property. Triazines33 have been reported to exhibit dihydro folate reductase inhibitory property. In continuation of our earlier work34 on 4(3H)-quinazolinones, the present study deals with the synthesis of a series of 6-bromo-2,3-disubstituted-4(3H)-quinazolinones by condensation of 6-bromo-2-substituted-benzoxazin-4-one with trimethoprim, pyrimethamine and lamotrigine. The chemical structures of the synthesized compounds were confirmed by means of IR, 1H-NMR, mass spectral and elemental analysis. The antiviral activity and cytotoxicity of the compounds were tested in E6SM (Herpes simplex-1 KOS, Herpes simplex-1 TK-KOS ACV, Herpes simplex-2 G, Vaccinia virus, Vesicular stomatitis virus, Parainfluenza-3 virus, Reovirus-1, Sindbis virus, Coxsackie virus B4 and Punta Toro virus) and HeLa cell culture (Vesicular stomatitis virus, Coxsackie virus B4 and Respiratory syncytial virus). Investigation of anti-HIV activity was done against replication of HIV-1 (HTLV-III B LAI) in MT-4 cells.

Chemistry The melting points were taken in open capillary tube on a Thomas Hoover melting point apparatus and are uncorrected. The IR spectra of the compounds were recorded on Bruker Vector-22 FT-IR with KBr pellets. 1H-NMR spectra were recorded on 500 MHz Bruker AMX 500 using DMSO-d6 as solvent. The chemical shifts are reported as parts per million downfield from tetramethylsilane (Me4Si). Mass spectra were recorded on Varian Atlas CH-7. Microanalyses for C, H, N were performed in Heraeus CHN Rapid Analyzer. All the compounds gave satisfactory chemical analyses (±0.4%). The purity of the compounds were checked by TLC on SiO2 gel (HF254: 200 mesh) coated glass plates using 4:1 CH3OH: CHCl3 as mobile phase and visualized by iodine vapoours.

Synthesis of 2-Methyl-6-bromo-3,1-benzoxazin-4-one A mixture of 5-bromoanthranilic acid (0.1 mol) and acetic anhydride (0.2 mol) was refluxed under anhydrous conditions for 4 h. The excess acetic anhydride was distilled off under reduced pressure. The product formed was filtered, vacuum dried and recrystallised using absolute ethanol. Yield = 58%, mp 180—181 °C. 1H-NMR (DMSO-d6) δ: 8.04 (s, 1H; 5-H), 7.67 (d, J = 6.2 Hz, 1H; 7-H), 7.48 (d, J = 6.2 Hz, 1H; 8-H), 1.41 (s, 3H; 2-CH3). IR (KBr) cm−1: 1686 (C=O), 1668 (C=N), 1143 (C-O-C), 811, 774 (Ar-H), 616 (C-Br). EIMS m/z: 240.19 (Caled for C10H8BrNO2: 240.06). Anal. Caled

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for $C_{18}H_{11}BrCl_{2}N_{6}O$: C, 51.14; H, 2.43; N, 13.62. Found: C, 53.87; H, 3.92; N, 12.26.

6-Bromo-2-methyl-3-[(4-amino-5-(4-chlorophenyl)-6-ethylpyrimidin-2-yl)]-4(3H)-quinazolinone (4): Yield = 84%, mp 194—195 °C. $^1$H-NMR (DMSO-$d_6$) $\delta$: 8.67 (s, 1H; 5-H), 8.18 (d, $J$ = 7 Hz, 1H; 8-H), 7.97 (d, $J$ = 7 Hz, 1H; 7-H), 7.49—7.65 (m, 9H; 2-$C_{6}H_{5}$ & $C_{6}H_{4}$), 3.43 (s, 2H; NH$_2$), 2.51 (q, $J$ = 5.9 Hz, 2H; $CH_{2}CH_{3}$), 2.23 (t, $J$ = 5.9 Hz, 3H; $CH_{2}CH_{3}$). IR (KBr) cm$^{-1}$: 3393 (NH$_2$), 1681 (C=O), 1657 (C=N), 833, 813, 711 (Ar–H), 590 (C–Br). El-MS $m/z$: 532.41 (Calcd for $C_{26}H_{19}BrClN_{5}O$: 532.87). Anal. Caled for $C_{26}H_{19}BrClN_{5}O$: C, 58.61; H, 3.59; N, 13.14. Found: C, 58.34; H, 3.68; N, 12.92.

6-Bromo-2-methyl-3-[(5-amino-6-(2,3-dichlorophenyl)-1,2,4-triazin-3-yl)]-4(3H)-quinazolinone (5): Yield = 82%, mp 240—241 °C. $^1$H-NMR (DMSO-$d_6$) $\delta$: 8.52 (s, 1H; 5-H), 8.14 (d, $J$ = 6 Hz, 1H; 8-H), 7.71 (d, $J$ = 6 Hz, 1H; 7-H), 7.41—7.66 (m, 3H; phenyl), 3.56 (s, 2H; NH$_2$), 2.15 (s, 3H; 2-CH$_3$). IR (KBr) cm$^{-1}$: 3455 (NH$_2$), 1681 (C=O), 1645 (C=N), 1438 (N=N), 830, 792, 737 (Ar–H), 604 (C–Br). El-MS $m/z$: 478.31 (Calcd for $C_{28}H_{17}BrClN_{4}O$: 478.13). Anal. Caled for $C_{28}H_{17}BrClN_{4}O$: C, 55.22; H, 2.32; N, 17.58. Found: C, 54.41; H, 2.04; N, 17.74.

6-Bromo-2-phenyl-3-[(4-amino-6-(2,3-dichlorophenyl)-1,2,4-triazin-3-yl)]-4(3H)-quinazolinone (6): Yield = 87%, mp 210—211 °C. $^1$H-NMR (DMSO-$d_6$) $\delta$: 8.82 (s, 1H; 5-H), 8.36 (d, $J$ = 7.1 Hz, 1H; 8-H), 8.08 (d, $J$ = 7.1 Hz, 1H; 7-H), 7.24—7.89 (m, 8H; 2-$C_{6}H_{5}$ & phenyl), 3.42 (s, 2H; NH$_2$). IR (KBr) cm$^{-1}$: 3393 (NH$_2$), 1679 (C=O), 1611 (C=N), 1435 (N=N), 827, 799, 781 (Ar–H), 608 (C–Br). El-MS $m/z$: 540.09 (Calcd for $C_{28}H_{17}BrClN_{4}O$: 540.21). Anal. Caled for $C_{28}H_{17}BrClN_{4}O$: C, 51.14; H, 2.43; N, 15.56. Found: C, 50.98; H, 2.63; N, 15.39.

**Antiviral Activity and Cytotoxicity** The antiviral activity and cytotoxicity of the synthesized compounds were
tested in E6SM (Herpes simplex-1 KOS, Herpes simplex-1 TK-KOS ACV, Herpes simplex-2 G, Vaccinia virus, Vesicular stomatitis virus, Parainfluenza-3 virus, Reovirus-1, Sindbis virus, Coxsackie virus B4 and Punta Toro virus) and HeLa cell culture (Vesicular stomatitis virus, Coxsackie virus B4 and Respiratory syncytial virus). (E)-5-(2-Bromovinyl)-2'-deoxyuridine, (S)-9-(2,3-dihydroxypropyl)adenine and (S)-9-(2,3-dihydroxypropyl)guanidine were used as standards. The minimum cytotoxic concentration (MCC) to cause a microscopically detectable alteration of normal cell morphology and minimum inhibitory concentration (MIC) to reduce virus-induced cytopathogenicity by 50% were recorded. The antiviral and cytotoxicity data are presented in Table 1 and 2.

**Anti-HIV Activity**

Cell Cultures: The MT-4 cells were grown in RPM1-1640 DM (“Dutch Modification”) medium (Flow Laboratories, Irvine, Scotland), supplemented with 10% (v/v) heat-inactivated fetal calf serum (FCS) and 20 μg/ml gentamicin (E. Merck, Darmstadt, F.R.G.). The cells were maintained at 37°C in a humidified atmosphere of 5% CO₂ in air. Every 3—4 d, cells were spun down and seeded at 3×10⁵ cells/ml in new cell culture flasks. At regular intervals, the MT-4 cells were analyzed for the presence of mycoplasm and consistently found to be mycoplasm-free.

Virus: HIV-1 (strain HTLV-IIIB LAI) was obtained from the culture supernatant of HIV-1 infected MT-4 cell lines. The Virus titer of the supernatant was determined in MT-4 cells. The virus stocks were stored at −70°C until used.

Anti-HIV Assay: Flat bottom, 96-well plastic microtiter plates (Falcon, Becton Dickinson, Mountain view, CA, U.S.A.) were filled with 100 μl 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× final test concentration) of compounds were added in 25 μl 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 was included for each compound.

50 μl 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×g and the supernatants were discarded. The MT-4 cells resuspended at 6×10⁵ cells/ml in a flask that was connected with an autoclavable dispensing cassette of Titertek multidrop dispenser. Under slight magnetic stirring 50 μl volumes were then transferred to the microtiter tray wells. The outer row wells were filled with 200 μl of medium. The cell cultures were incubated at 37°C 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.

MTT Assay: The MTT assay was based on the reduction of the yellow colored 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma Chemical Co., St. Louis, MO, U.S.A.) by mitochondrial dehydrogenase of metabolically active cells to a blue formazan which can be

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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 °C in a CO2 incubator for one hour. A fixed volume of medium (150 μl) was then removed from each cup using M96 washer (ICN flow) without disturbing the MT-4 cells clusters containing the microtiter plates.

Solubilization of the formazan crystals was achieved by adding 100 μl of 10% 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 an eight-channel computer-controlled photometer (Multiskan plate shaker) at two wavelengths (540, 690 nm). The absorbance at 540 nm. The dose achieving 50% protection according to the above formula was defined as the 50% effective concentration. Azidothymidine was used as standard. The anti-HIV activity data are presented in Table 2.

RESULTS AND DISCUSSION

6-Bromo-2-phenyl-3-[(4-amino-5-(4-chlorophenyl)-6-ethylpyrimidin-2-yl]-4(3H)-quinazolinone (4) exhibited the most potent antiviral activity with a MIC of 1.92 μg/ml against vaccinia virus in E6SM cell culture. The other compounds did not exhibit antiviral activity nor afford significant cytoprotection to the E6SM and HeLa cell culture when challenged with the viruses. The study implies that 4 may possess activity against Pox viruses including variola. In the anti-HIV study, 6-bromo-2-methyl-3-[(4-amino-5-(4-chlorophenyl)-6-ethylpyrimidin-2-yl]-4(3H)-quinazolinone (3) and 6-bromo-2-phenyl-3-[(4-amino-5-(4-chlorophenyl)-6-ethylpyrimidin-2-yl]-4(3H)-quinazolinone (4) exhibited the least cytotoxic concentration (0.424, 0.461 μg/ml) which is an index of the infective viability of mock infected MT-4 cells with HIV-1. None of the compounds exhibited significant anti-HIV activity.

REFERENCES

13) Spirkova K., Stankovsky S., Mrvova A., Cipak L., Chem. Pap., 53,