Synthesis, anti-HIV activity and molecular modeling study of some new pyrimidine analogues

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1. Introduction

Pyrimidine and its derivatives demonstrated a diverse array of biological and pharmacological activities including antitumor [1-6], antimicrobial [7-10], and antihypertensive [11] in addition to their cardiovascular [12,13] and diuretic [14,15] properties. Some pyrimidine analogues exhibited potent antiviral activity against a wide spectrum of unrelated viruses, such as poliovirus [16] and herpes virus [17] and anti-HIV agents [18-20], whereas two recent diarylpyrimidines (DAPY), rilpivirine 1 [21] and etravirine 2 [22,23] have been classified as non-nucleoside reverse transcriptase inhibitors (NNRTIs), meanwhile Chen et al. [24] have reported a new class of diarylpyrimidines (CHX-DAPYs) as potent NNRTI's. Further, several pyrimidine derivatives exhibited significant antitumor activity e.g. imatinib mesylate (Gleevec, 3) [25], an interesting novel agent for the treatment of chronic Leukemia is the tyrosine kinase inhibitor which contains a 4-pyridyl substituted pyrimidine-2-amine, in addition to a 2,4-diamino-\(N^6\)-6-diaryl-pyrimidines where the latter were identified to block the proliferation of tumor cell lines in \textit{vivo}, especially duodenum cancer [26]. Monastrol 4 [27] is another model of pyrimidine derivative as inhibitor of kinesin Eg5 that interact with microtubule and then causes mitotic arrest [28] (Figure 1). Recently, Kim et al. [29] have reported some novel pyrimidine derivatives as potent acid pump antagonists (APAs). Jian et al. [30] have reviewed the biological and medicinal significance of pyrimidines extensively.

\begin{center}
\includegraphics[width=0.5\textwidth]{pyrimidine_analogues.png}
\end{center}

\textbf{Figure 1.} Pyrimidine analogues antitumor and anti-HIV drugs.
In continuation of our ongoing work on the synthesis of new anti-HIV pyrimidine derivatives [31,32], we report here the synthesis of a new series of pyrimidines having substituted amino and thio groups with evaluation of their anti-HIV activity as well as the SAR and molecular modeling study.

2. Experimental

2.1. Instrumentation

Melting points are uncorrected and were measured on a Büchi melting point apparatus B-545 (Büchi Labortechnik AG, Switzerland). NMR data were obtained on 400 and 600 MHz (400 MHz and 1H) and 159.01 MHz (13C) spectrometers (Avance III, Bruker, Germany) with TMS as internal standard and on the δ scale in ppm. Heteronuclear assignments were verified by 1H, 13C HMBC and 1H, 13C HSQC NMR experiments. Microanalytical data were obtained with a Vario, Elemental analyzer (Shimadzu, Japan). Analytical silica gel TLC plates 60F254 were obtained from Merck. Microwave supported reaction was performed in a SmithSynthesizer (temperature control of irradiation power up to 800 W). All reagents were obtained from commercial suppliers and were used without further purification.

2.2. Synthesis

2.2.1. General procedure for the preparation of 2,6-diamino-4-alkylamino-5-(p-bromophenyl)diazenyl)pyrimidine derivatives (6-13)

A solution of compound 5 (164 mg, 0.50 mmol) in DMF (20 mL) and an appropriate amine (1.00 mmol) was heated in an oil bath at 90-100 °C for 4-5 h. Then water (25 mL) was added, the solution was cooled, and the yellow precipitate was collected, washed with water, and dried. Recrystallization from ethanol afforded the desired product (Scheme 1).

2,6-Diamino-4-benzylamino-5-p-bromophenylazopyrimidine (6): From benzylamine (107 mg). Yield: 110 mg (55%). M.p.: 160-167 °C. Rf = 0.75. 1H NMR (400 MHz, DMSO-d6, δ, ppm): 8.84-8.14 (br s, 2H, NH2), 7.72 (d, 2H, J = 7.9 Hz, H-Ar), 7.54 (d, 2H, J = 7.9 Hz, H-Ar), 7.36-7.24 (m, 6H, H-Ar + CH2-NH2), 6.62 (br s, 2H, NH2), 4.72 (d, 2H, J = 6.5 Hz, CH2). 13C NMR (159.01 MHz, DMSO-d6, δ, ppm): 163.3 (C2(pyrimidin)), 161.0 (C4(pyrimidin)), 152.0 (C6(pyrimidin)), 139.0, 131.7, 128.3, 127.3, 126.9 (Carom), 122.7 (C-Br), 119.1 (Cgen), 111.0 (C5(pyrimidin)), 42.9 (CH2). Anal. calc. for C19H17BrN2: C, 51.27; H, 4.05; N, 24.62. Found: C, 51.04; H, 3.92; N, 24.41.

2,6-Diamino-4-(p-bromophenyl)diazenyl)-4-(2-picolyl amino)pyrimidine (7): From 2-picolylamine (108 mg). Yield: 64 mg (32%). M.p.: 193-196 °C. Rf = 0.54. 1H NMR (400 MHz, DMSO-d6, δ, ppm): 8.50 (d, 2H, J = 4.8 Hz, NH2), 8.36 (d, 1H, J = 8.2 Hz, Hpicolyl-6'-Ar), 7.78 (m, 3H, H arom-3' + H arom-5' + Hpicolyl-4'), 7.55 (d, 2H, J = 8.2 Hz, Hpicolyl-3' + Hpicolyl-5'), 7.31 (d, 2H, J = 8.0 Hz, H arom-2' + H arom-6'), 6.60 (br s, 2H, NH2), 4.73 (d, 2H, Jpicolyl = 6.1 Hz, Hpicolyl-2), 3.28 (d, 1H, HcH3). 13C NMR (150.91, DMSO-d6, δ, ppm): 163.2 (C2(pyrimidin)), 161.5 (C4(pyrimidin)), 157.6 (C2(pycolyl)), 152.0 (C6(pyrimidin)), 149.5 (C6(pycolyl)), 141.6 (C4(pycolyl)), 131.7, 122.9, 122.1 (Cgen), 119.3 (C3(pycolyl) + C5(pycolyl)), 110.1 (C5(pyrimidin)), 40.0 (NMe2). Anal. calc. for C41H29BrN4: C, 48.13; H, 3.79; N, 28.07. Found: C, 47.91; H, 3.68; N, 27.81 %.

Scheme 1
2,6-Diamino-5-((p-bromophenyl)diazényl)-4-piperazino-pyrimidine (13): From piperazine (86 mg). Yield: 153 mg (81%). M.p.: 236-242 °C. \( R_f = 0.11 \). \( ^1H \) NMR (400 MHz, DMSO-\( d_6 \), ppm): 0.71 (d, 2H, J = 7.7 Hz, H arom-3' + H arom-5'), 3.13 (br s., 2H, H pyrimid-4'), 6.66 (br s., 2H, H NH), 3.30 (br s., 1H, NH), 3.09 (br s., 4H, 2xCH2piperazin). \( 13C \) NMR (150.91, DMSO-\( d_6 \), ppm): 49.6 (2xCH2piperazin). Anal. calc'd. for C14H17BrN8: C, 44.46; H, 4.47; N, 25.69%.

2,4-Diamino-5-((p-bromophenyl)diazényl)-6-((chlorooxy)ethylthio)pyrimidine (16): From 4-chlorobenzylthiol (143 mg). Yield: 74 mg (53%). M.p.: 232-236 °C. \( R_f = 0.55 \). \( ^1H \) NMR (400 MHz, DMSO-\( d_6 \), ppm): 9.26 (br s., 2H, NH), 8.19 (br s., 2H, NH), 7.75-7.29 (m, 8H, H arom), 4.36 (s, 2H, CH2). \( 13C \) NMR (150.91, DMSO-\( d_6 \), ppm): 164.6 (C(4)pyrimid.), 161.1 (C(2)pyrimid.), 155.9 (C(6)pyrimid.), 132.2, 132.0, 128.4, 123.2 (C arom.), 122.1 (C-Br), 118.6 (C(5)pyrimid.) 31.2 (CH3). MS ([+]-FAB): \( m/z = 433 \) [M]+. Anal. calc'd. for C14H15BrC2N4S: C, 45.40; H, 3.14; N, 18.69. Found: C, 45.21; H, 3.02; N, 18.42%.

2.2.3. Reaction of compound 17 and 18 with thiourea under MWI

A crushed mixture of compound 17 (100 mg, 0.46 mmol) or 18 (100 mg, 0.71 mmol) and thiourea (10 mg, 1.31 mmol) was irradiated in a microwave oven (800 W) for 25 min. The mixture was poured into ice-cold water and the solid residue was washed with ether followed by a little amount of cold ethanol. Recrystallization from ethanol afforded the desired product (Scheme 3).

2-Amino-6-(benzoxyl)pyrimidine-4-thione (19): Yield: 76 mg (71%). M.p.: 136-138 °C. \( R_f = 0.57 \). \( ^1H \) NMR (400 MHz, DMSO-\( d_6 \), ppm): 7.42-7.33 (m, 5H, H arom), 6.15 (s, 1H, NH), 5.31 (s, 1H, Hpyrimid-5'), 5.13 (s, 2H, CH2). \( 13C \) NMR (150.91, DMSO-\( d_6 \), ppm): 184.9 (C=O), 171.8 (C(4)pyrimid.), 153.8 (C2)pyrimid.), 137.1 (C(pyr), 128.9, 128.8, 128.4, 128.2, 128.2 (C arom.), 124.7 (C(5)pyrimid.), 65.7 (CH3). Anal. calc'd. for C5H7N3OS: C, 56.63; H, 4.75; N, 18.01. Found: C, 56.41; H, 4.65; N, 17.82%.

2-Amino-6-methylthiopirimidine-4-thione (20): Yield: 76 mg (67%). M.p.: 189-205 °C. \( R_f = 0.37 \). \( ^1H \) NMR (400 MHz, DMSO-\( d_6 \), ppm): 7.28 (br s., 2H, NH), 6.76 (s, 1H, NH), 5.35 (s, 1H, Hpyrimid-5'), 4.07 (s, 3H, OCH3). \( 13C \) NMR (150.91, DMSO-\( d_6 \), ppm): 184.9 (C=O), 171.8 (C(4)pyrimid.), 153.8 (C2)pyrimid.), 146.2 (C(pyr), 119.0 (C arom.), 51.7 (OCH3). Anal. calc'd. for C3H3N2OS: C, 59.0; H, 4.28; N, 26.52%.

2.2.4. Synthesis of 2-(2-Amino-6-methylthiopirimidin-4-yl) mercaptoacetic acid (21)

To a solution of compound 20 (55 mg, 0.34 mmol) in DMF (5 mL) containing potassium carbonate (30 mg) was added 2-mercaptoacetic acid (30 mg, 0.32 mmol) and stirred at room temperature for 16 h. After cooling, the mixture was filtered and the filtrate was evaporated to dryness and the residue was purified on a short column of SiO2. To a solution of compound 21 (35 mg, 47%) (Scheme 3). M.p.: 142-154 °C. \( R_f = 0.1 \). \( ^1H \) NMR (400 MHz, DMSO-\( d_6 \), ppm): 10.95 (s, 1H, OH), 7.23 (br s., 2H, NH), 6.33 (s, 1H, Hpyrimid-5'), 4.08 (s, 2H, CH2), 3.88 (s, 3H, OMe). \( 13C \) NMR (150.91, DMSO-\( d_6 \), ppm): 71.7 (CO2H), 170.6 (C(6)pyrimid.), 168.5 (C(4)pyrimid.), 155.9 (C(2)pyrimid.), 94.6 (C(5)pyrimid.), 51.9 (OMe), 31.0 (CH3).
2.2.5. Reaction of compound 21 with sodium hypochlorite and ammonium hydroxide

Compound 21 (104 mg, 0.66 mmol) or 20 (54 mg, 0.34 mmol) was added to a solution of sodium hypochlorite (5 mL) and the reaction mixture was stirred at 60 °C for 4 h. Ammonium hydroxide (10 mL) was added to the reaction mixture, and stirred for an additional 1 h at room temperature, the precipitate was filtered and washed with water several times and dried, followed by washing with dry ether to give compound 22 or 23, respectively (Scheme 3).

2-Amino-4-(aminothio)-6-(benzoxo)pyrimidine (22): Yield: 111 mg (69%). M.p.: 286-289 °C; Rf = 0.32. 1H NMR (400 MHz, DMSO-d6, δ, ppm): 7.43-7.33 (m, 5H, H arom.). 2.73 1H (br s., 2H, NH2). 6.14 (s, 1H, Hpyrimid.). 5.39 [br s., 2H, SNH2]. 3.51 [s, 2H, CHs]. 13C NMR (150.91, DMSO-d6, δ, ppm): 189.4 (CSNH2), 166.1 (C4(pyrimid.)), 160.5 (C2(pyrimid.)). 129.0-126.9 (C arom.), 100 (C5(pyrimid.)). Anal. calcd. for C11H12N4OS: C, 53.21; H, 4.87; N, 22.56. Found: C, 52.98; H, 4.70; N, 22.68 %.

2-Amino-4-(aminothio)-6-methoxo)pyrimidine (23): Yield: 36 mg (61%). M.p.: 280-291 °C; Rf = 0.43. 1H NMR (400 MHz, DMSO-d6, δ, ppm): 7.41 (br s., 2H, NH2), 5.99 (s, 1H, Hpyrimid-5), 3.87 [s, 3H, OMe]. 2.31 [s, 2H, SNH2]. 13C NMR (150.91, DMSO-d6, δ, ppm): 189.0 (C6(pyrimid.)), 167.8 (C4(pyrimid.)), 153.2 (C2(pyrimid.)), 94.9 (C5(pyrimid.)), 57.0 (Ome). Anal. calcd. for C9H14N4O2S: C, 34.87; H, 4.68; N, 32.53. Found: C, 34.59; H, 4.55; N, 32.33 %.

2.2.6. Synthesis of 2-amino-6-methoxy)pyrimidine-4-sulfonamide (24)

A suspension of compound 23 (80 mg, 0.46 mmol) in H2O2 (5 mL) was stirred at room temperature for 4 h. The solution was evaporated to dryness and the residue was washed with water (3 x 10 mL), dried and then with ether. The dried crude product was purified on a short SiO2 column (5 g) using chloroform:methanol (4:1, v/v) as eluent, to give compound 24 (70 mg, 74%) (Scheme 3). M.p.: 156-158 °C; 1H NMR (400 MHz, DMSO-d6, δ, ppm): 7.32 (br s., 2H, NH2). 6.51 [s, 1H, Hpyrimid-5], 3.98 [s, 3H, OMe]. 2.11 (br s., 2H, SO3NH2). 13C NMR (150.91, DMSO-d6, δ, ppm): 168.9 (C6(pyrimid.)), 161.5 (C4(pyrimid.)), 154.7 (C2(pyrimid.)), 96.2 (C5(pyrimid.)), 57.2 (Ome). Anal. calcd. for C11H14N4O2S: C, 39.41; H, 3.95; N, 27.44. Found: C, 39.19; H, 3.86; N, 27.21 %.

3. Results and discussion

3.1. Chemistry

Recently, reported the synthesis of azopyrimidine 5 [33], and selected here as a starting material for the synthesis of new azopyrimidine analogues having alkyl and aryl amino groups at C-4. Thus, compound 5 was subjected to a nucleophilic displacement of chlorine group by treatment with various amines in DMF at 90-100 °C for 4.5 h, leading to the new derivatives 6-13 in good to moderate yields (55-89 %), except the products 7 and 10 were obtained in a lower yields 32 and 36%, respectively (Scheme 1), might due to the steric factor (Scheme 1).

The structures of 6-13 were established by 1H, 13C NMR and mass spectral data. In the 1H NMR spectra, both amino groups at C-2 and C-6 of pyrimidine backbone appeared almost at the same regions as broad singlets at δ 6.84-6.38 ppm and δ 10.61-7.79 ppm, respectively, assigned by D2O exchange. The amino groups at C-6 of the analogue 6 showed two broad singlets (δ 8.85 and 8.14 ppm) and not doublets as expected, might be due to the hydrogen bonding with the nitrogen of the azo group at C-5. The aromatic and the aliphatic protons were fully analyzed (cf. Experimental section).

Next, the analogue 5 was used as a precursor for the synthesis of new 5-arylazo-4-ethyl[aryl]thio-pyrimidine derivatives to examine their antiviral activity in comparison to the azoaryl analogues 6-13. Thus, treatment of compound 5 with sodium ethanethiolate, sodium thiophenolate, or p-chloro-benzylkohol in DMF afforded, via nucleophilic displacements of the chlorine group, 14-16 in 74, 68 and 53% yield, respectively (Scheme 2).

The structures of compounds 14-16 were assigned by the 1H and 13C NMR spectra. The 1H NMR spectra showed rather similar patterns for the phenyl and ethyl protons. The methylene and methyl protons of SET group appeared as quartet and triplet at δ 3.99 and 1.36 ppm (J = 7.1 Hz), respectively, whereas the aromatic protons were fully analysed (cf. Experimental section).
In the $^{13}$C NMR spectra of compounds 14-16, C-4, C-5 and C-6 together with the aromatic carbon atoms were identified. However, compound 14 has been selected for further NMR experiment. In the gradient-selected HMBC spectrum [34] of compound 14, C-4 of the pyrimidine ring at $\delta$ 163.0 ppm showed a $^{1}J_{CH}$ coupling with the proton of CH$_{2}$CH$_{2}$SO$_{2}$NH$_{2}$ group at $\delta$ 3.99 ppm, in addition to a $^{2}J_{CH}$ coupling with the methyl protons of the CH$_{2}$CH$_{2}$SO$_{2}$NH$_{2}$ group at $\delta$ 1.23 ppm. Further, the aromatic protons H-3 and H-5 at $\delta$ 7.72 ppm revealed two $^{3}J_{CH}$ couplings with C-4 (C-Br) of the aromatic ring at $\delta$ 121.6 ppm, while the same carbon atom (C-Br) showed two $^{3}J_{CH}$ couplings with H-2 and H-6 of the same ring at $\delta$ 6.68 ppm (Figure 2).

Our work was modified by conversion of the chloro residue in the pyrimidine scaffold to the thione, following Lawson and Tankler method [35]. Thus, compound 17 and 18 were treated with thiourea under microwave irradiation (MWI) in a free solvent condition for 15 min after purification, the pyrimidine-thione analogues 19 and 20 in 71 and 67 % yield, respectively. Treatment of compound 20 with 2-mercaptacetic acid in DMF in the presence of K$_{2}$CO$_{3}$ at room temperature for 16 h afforded regioselectively [36], after chromatographic purification, 4-thio-ethylacetic acid derivative 21 (47 %).

Next, treatment of compound 19 and 20 with sodium hypochlorite followed by ammonium hydroxide, following Rice et al. method [37], furnished after purification, the 4-aminothio-pyrimidine derivatives 22 and 23 in 61 and 68% yield, respectively. Oxidation of compound 23 with H$_{2}$O$_{2}$ at room temperature afforded, after chromatographic purification, the sulfoximine 24 in 74% yield (Scheme 3).

The structures of compounds 19-24 were elucidated from their $^{1}$H and $^{13}$C NMR spectra. In the $^{1}$H NMR spectra of compounds 19 and 20, H-5 of the pyrimidine ring were appeared at $\delta$ 5.31 and 5.39 ppm, respectively, whereas the protons of the amino groups at C-2 were resonated as broad singlet at $\delta$ 7.07 and 7.33 ppm, respectively. The SN$_{2}$ protons of compound 22 resonated as a broad singlet at $\delta$ 5.39 ppm, while the singlet and broad singlet at $\delta$ 6.76 and 2.31 ppm were assigned to NH proton of the pyrimidine thione 20 and the NH$_{2}$ protons of C-4 of the analogue 23, respectively. The singlets at $\delta$ 5.35 and 5.99 ppm were attributed to H-5 of the pyrimidine ring, in addition to two singlets at $\delta$ 4.07 and 4.09 ppm, assigned to the methoxy group at C-6 of compound 20 and 23, respectively. Compound 24 showed singlet and broad singlet at $\delta$ 6.51 and 2.11 ppm, identified as H-5 and SO$_{2}$NH$_{2}$ protons, respectively. In the $^{13}$C NMR of compound 19-24, the resonances at the lower field regions $\delta$ 189.4-168.9 ppm were assigned to C-6 (C-S) of the pyrimidine ring in comparison for those of the starting materials 17 and 18 at $\delta$ 164.1 and 162.7 ppm, respectively. C-2, C-4 and C-5 together with the substituting carbon atoms were fully identified (of Experimental section). All the synthesized were confirmed also from their $^{1}$H, $^{13}$C HSQC [38] NMR spectra.

3.2. In vitro anti-HIV activity

Compounds 6-13, 14-16 and 19-24 were tested for their in vitro anti-HIV-1 (strain IIIa) and HIV-2 (strain ROD) activity in human T-lymphocyte (MT-4) cells based on an MTT assay [39]. The results are summarized in Table 1, in which the data for nevirapine (BOE/BIRG587) [40] and azidothymidine (DDN/AZT) [41] were included for comparison.

Table 1. In vitro anti-HIV-1 and HIV-2 of new pyrimidine analogues 4-16 and 19-27.

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<th>CC$_{50}$ (µg/mL)</th>
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* SI: selectivity index (EC$_{50}$/CC$_{50}$).

Compounds-induced cytotoxicity was also measured in MT-4 cells parallel with the antiviral activity. All compounds are inactive except compounds 14-16 and 21 which showed EC$_{50}$ values of > 2.12, 1.99, 1.80 and 1.92 µg/mL, respectively, but no selectivity was observed (SI < 1).
From the SAR analysis, we found that the alkyl- or arylthio substituents at C-4 of the pyrimidine ring, e.g., compounds 14-16 or thioalkyl acetic acid moiety at C-6 of the same ring, e.g.: compound 21 were well tolerated in the hydrophobic region of HIV-RT and then showed higher activity than those of the alkyl- or arylamino substituents at C-4 of the same ring; e.g.: compounds 6-13, and resulted in loss of activity. This means that the thio groups targeting the hydrophobic binding pocket of HIV-1 RT.

3.3. Molecular modeling analysis

The molecular docking was performed using SYBYL-X 1.1 and the docking results were shown by PyMOL [42]. Our molecular docking analysis of the new analogues based on the modeling study, which was performed to understand the binding mode of these analogues with the HIV-RT binding pocket [NNIPB] (PDB code: 3DLG, [43]). Compound 15 has been selected for the docking modeling study, since its binding energy score -8.01, indicating a selectivity and potency profiles of substituted aryl-azopyrimidine to bind the active site of HIV-RT pocket (Figure 3). As shown in figure 3, the aromatic rings of compound 15 fitted into an aromatic rich subpocket surrounded by the aromatic side chains of Tyr179 and Tyr186. The pyrimidine backbone was located in the middle of the binding pocket, anchoring the phenylthio substituent at C-4 in a favourable position for hydrogen bonding with the OH group of Tyr 186 and other two hydrogen bonding of amino group at C-6 and azo group (N=N) at C-5 with Lys103 of the RT enzyme. Overall, the combination of hydrophobic interaction and π-stacking appears to govern the binding of compound 15 with HIV RT.

4. Conclusion

In conclusion, synthesis of new 2,6-diamino-4-alkylamino-5-p-bromophenylazopyrimidine derivatives, 6-13, the corresponding 4-ethyl- and arylthio analogues, 14-16, and the 4-thio derivatives 19-25 has been described. All the new synthesized compounds have been evaluated for their activity against HIV-1 and 2. Compounds 14-16 and 21 exhibited potential activity against HIV-1, whereas the others analogues shown moderate to poor activity. Compound 15 have been selected for the molecular modeling study showing its binding to the reverse transcriptase enzyme pocket through three hydrogen bonding and two hydrophobic interactions.

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References


